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QUANTITATIVE ANALYSIS, BY GAS–LIQUID CHROMATOGRAPHY AND MASS FRAGMENTOGRAPHY, OF MONOSACCHARIDES AFTER METHANOLYSIS AND DEAMINATION

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ABSTRACT

A method has been developed for the quantitative analysis, by g.l.c. and g.l.c.–m.s., of monosaccharides commonly appearing in glycoconjugates. It involves liberation of the component sugars, *N*-deacetylation by methanolysis, nitrous acid deamination of amino sugars and sialic acid, and g.l.c. or g.l.c.–m.s. analysis as their trimethylsilyl ethers. The method has been tested on a crude, lipid-free, protein fraction obtained from rat brain. The structure of the main deamination product of sialic acid has been characterized.

INTRODUCTION

Gas–liquid chromatography (g.l.c.) has provided a sensitive, precise, and rapid method for quantitation and identification of monosaccharides, as well as certain di- and tri-saccharides, and extensive articles on this topic have been published[1,2]. Combined gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.), which has increasingly been used for structural and quantitative analysis, has proved to be a method of choice when picomole amounts of carbohydrate are to be quantitated[3–5], but no method for a single-step analysis for all of the common monosaccharides has been thus far available.

Liberation of component sugars from glycoconjugates by use of aqueous acid catalysis has been considered to be the main cause of loss in carbohydrate analysis[6]. Methanolysis, where the liberated monosaccharides are converted into the corresponding methyl glycosides, results in little sugar decomposition, and makes possible[7] the analysis of sialic and uronic acids. Because, simultaneously, amino sugars and sialic acid lose their *N*-acetyl groups, an *N*-reacetylation step is employed[7,8] for good separation of amino sugars in g.l.c.; this, however, restores their marked polarity, and increases their tendency towards adsorption, making quantitative analysis of them in small amounts difficult.

Nitrous acid deamination of *N*-deacetylated 2-amino sugars is a highly selective

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reaction for the cleavage of glycosidic linkages next to them, with the formation[9] of 2,5-anhydrohexoses.

When used after methanolysis, this reaction completes the liberation of amino sugars from potential unhydrolyzed, *N*-deacetylated oligosaccharides having a high resistance to cleavage by acidic methanol. 2,5-Anhydrohexitols are readily analyzed by g.l.c., and, because of their considerably lower polarity, compared to 2-acetamido-2-deoxyhexoses, they appear as peaks having retention times shorter than those of the methyl glycosides of neutral hexoses[10,11].

The object of this work was to develop a method for the quantitative analysis by g.l.c. and g.l.c.–m.s. of mixtures of monosaccharides at the picomole level, and to investigate their concentrations in less-pure material of biological origin. Methods for methanolysis[7], nitrous acid deamination[9,10], and per(trimethylsilyl)ation[8] were adapted for this purpose.

EXPERIMENTAL

Materials. — All monosaccharides were purchased from the Sigma Chemical Company, St. Louis, MO, U.S.A. Deuterated (98% D atoms), algal-sugar mixture was from MSD, Sharp & Dohme GmbH, Munich, GFR. *myo*-Inositol, or deuterated algal-sugar mixture, or both, was used as the internal standard, and was added to the sample tubes before the methanolysis step.

Methanolysis. — Samples were prepared for the assay procedure by methanolysis[7]. A dry sample was dissolved in 500 μL of 0.5M methanolic HCl containing 1.5% of H_2O, and the solution was kept for 18 h at 85°. For the protein samples, 1.0M methanolic HCl for 18 and 24 h at 85° was also used. The samples were cooled, made neutral with silver carbonate (200 mg), centrifuged, and the supernatant liquors decanted into other tubes. The precipitates were washed twice with 500 μL of methanol, and the supernatant liquors were collected, and evaporated under nitrogen.

Deamination. — Distilled water (50 μL), 1:2 acetic acid–water (50 μL), and 5.5M aqueous sodium nitrite solution (20 μL) were added to the dried samples, and the mixture was shaken intermittently for 30 min at room temperature. The excess of sodium was removed by adding a suspension (100 μL) of Dowex-50 X2 (H^+) resin (40%, w/v, in distilled water), and intermittent mixing was continued for[10] another 30 min. The contents of the tubes were allowed to drain through small pipet columns containing Dowex-50 X2 (H^+) resin (~500 μL) on glass-wool plugs. The columns were eluted three times with 1:1 (v/v) methanol–water, and the eluates were combined, and evaporated under nitrogen.

Reduction, remethanolysis, and (trimethylsilyl)ation. — Samples were dissolved in distilled water (50 μL), and reduced with 0.22M sodium borohydride (50 μL) for[10] 1 h at room temperature. The excess of borohydride was decomposed by addition of glacial acetic acid (20 μL), and the samples were evaporated under nitrogen.

Borate was removed by three additions, and evaporations to dryness, of acidic methanol [methanol (300 μL) containing glacial acetic acid (20 μL)].

The partially hydrolyzed methyl ester groups of deaminated sialic acid were re-esterified by a short methanolysis step with 0.5M methanolic HCl during 1 h at 80°, followed by neutralization as already described. Dried samples were per(trimethylsilyl)ated with 1:2:10 chlorotrimethylsilane–hexamethyldisilazane–pyridine for 30 min at room temperature, the solutions evaporated, and the residues dissolved in hexane and introduced into g.l.c. and g.l.c.–m.s. columns.

Analysis of protein samples. — The crude, protein fraction from a 2-month-old, male, Albino Wistar rat-brain was used[3]. The tissue was homogenized in 2:1 (v/v) chloroform–methanol (20 vol.) at 4°, centrifuged for 30 min at 20,000*g*, the tissue homogenized in 1:2 (v/v) chloroform–methanol (10 vol.), and centrifuged as before[12]. The lipid-free precipitate was used for further analysis.

Gas–liquid chromatography and gas–liquid chromatography–mass spectrometry. — Mass spectra were recorded with a Varian MAT CH-7 instrument equipped with a Varian Aerograph 1700 gas chromatograph and a SpectroSystem 100S computer system. Mass-fragmentographic detection was achieved with an Altema AL 5 multiple-ion detector. The ionization potential was 70 eV, and the ionization current 300 μA. Glass columns (2 m × 2 mm, i.d.) filled with 2.2% of SE-30 on Gas Chrom Q (100–120 mesh) (from Applied Science Laboratories, State College, PA) were used at an initial temperature of 120° with temperature programming at the rate of 4°.min^{-1} to a final temperature of 250°. For g.l.c., a Carlo Erba Fractovap Model 2151 AC gas chromatograph fitted with a capillary glass column (25 m × 0.25 mm i.d.) was used under the same conditions. The columns, coated with OV-101, were purchased from Prolab, Helsinki, Finland.

RESULTS AND DISCUSSION

I. General course of the analysis (see Fig. 1)

Methanolysis. This liberates monosaccharides from glycoconjugates, converting

Native sugar:	neutral hexose	acetamido sugar	sialic acid
	↓	↓	↓
Methanolysis:	methyl glycoside	*N*-deacetylated methyl glycoside	*N*-deacetylated methyl ester methyl glycoside
		↓	↓
Nitrous acid deamination:	methyl glycoside	2,5-anhydrohexose	nonulosonic acid methyl ester methyl glycoside
		↓	
Borohydride reduction:	methyl glycoside	2,5-anhydrohexitol	n.d.[a]
Mild methanolysis:	methyl glycoside	2,5-anhydrohexitol	nonulosonic acid methyl ester methyl glycoside

Trimethylsilylation, and introduction into the gas–liquid chromatograph.
[a]n.d. = not detected.

Fig. 1. Flow-sheet of procedure for quantitative determination of monosaccharides by g.l.c.–m.s.

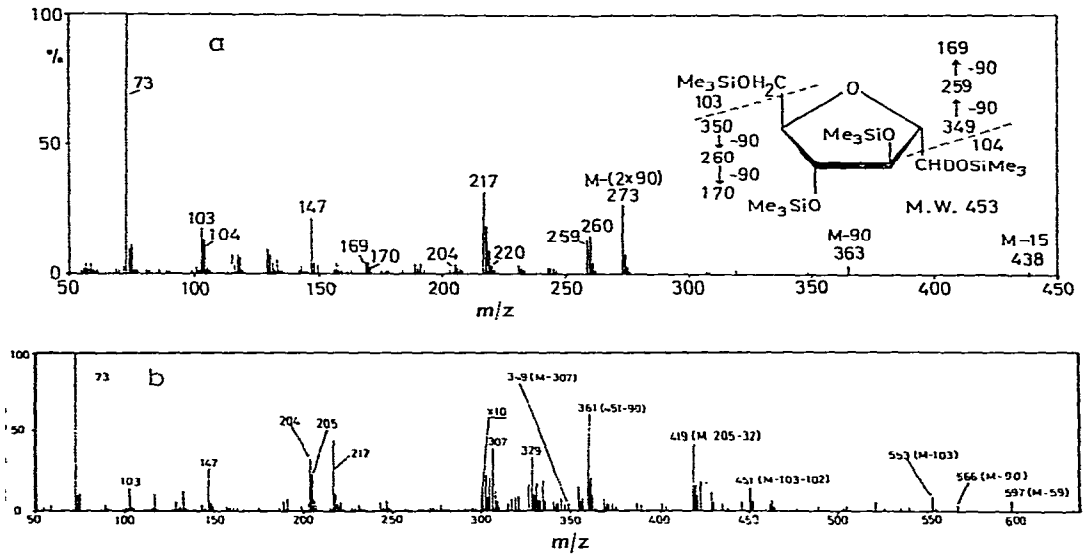


Fig. 2. (a) The mass spectrum and major fragments of 2,5-anhydro-D-mannitol after borodeuteride reduction of C-1, as its Me_3Si derivative. (b) The mass spectrum of the major product of deamination of sialic acid methyl ester glycoside [methyl (methyl 3-deoxy-D-*glycero*-D-*galacto*-nonulosid)onate] as its Me_3Si derivative.

hexoses and amino sugars into their methyl glycosides, and sialic acid into its methyl ester methyl glycoside[1]. Methanolic hydrochloric acid (0.5M) for 16 h at 85° was also sufficient to *N*-deacetylate, completely, the standard, monosaccharide mixture, and this was confirmed by g.l.c. When applied to glycoconjugates of biological origin, M methanolic hydrochloric acid for 18–24 h at 85° was used, to ensure complete liberation and *N*-deacetylation of the monosaccharides. When >M acids, or temperatures >85° were used, there was no significant increase in the release of amino sugars, but increased decomposition of neutral monosaccharides and sialic acid was observed by g.l.c.–m.s. These findings are in good agreement with those of Chambers *et al.*[7]. The internal standards were added to the test tubes before methanolysis, in order to subject them to the same conditions as were applied to the sample.

Deamination. Nitrous acid deamination of 2-amino-2-deoxyhexoses is a highly selective, mild reaction leading to the formation of 2,5-anhydrohexoses[9]. A characteristic feature of the reaction is the cleavage of the glycosidic linkages of the amino sugars. When methanolysis degrades glycosidic linkages by unspecific, acid catalysis, deamination completes the liberation of sugars from potential *N*-deacetylated oligosaccharides, which have high resistance to cleavage by acidic methanol. In this way, all glycosidically linked, *N*-deacetylated amino sugars are liberated under mild conditions, and, together with neuraminic acid, can readily be analyzed by g.l.c. The N–glycosyl linkage between asparagine and 2-acetamido-2-deoxy-D-glucose is, however, quite stable under these conditions[13]. The glycosidic methyl groups, formed on methanolysis of the amino sugars, are cleaved off as the 2,5-anhydrohexoses are

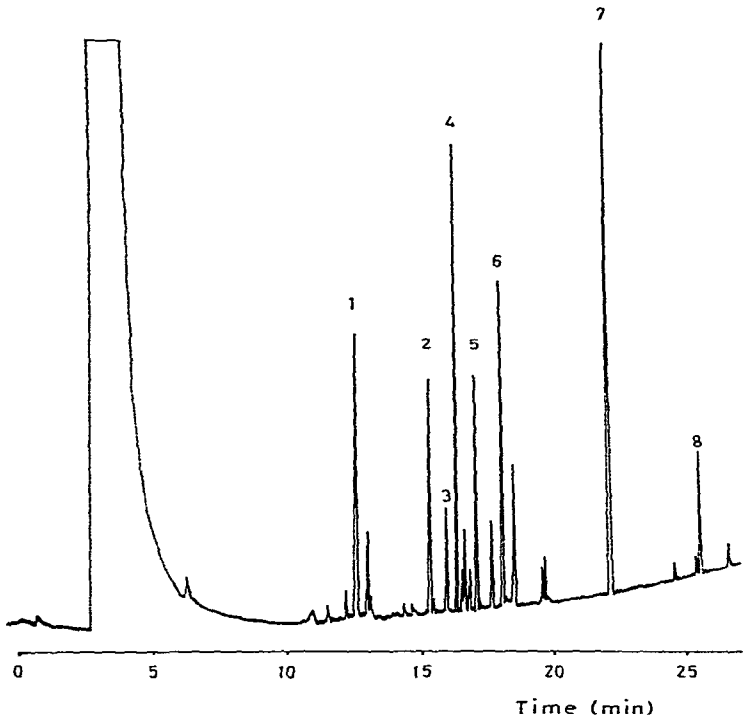


Fig. 3. A gas–liquid chromatogram of a standard, sugar mixture. (The analysis was performed in a 25-m long, open, glass capillary column, coated with OV-101. An initial temperature of 120° and a final temperature of 250°, with temperature programming at 4°.min^{-1}, were used. The peaks are numbered as in Table II.)

formed (see Fig. 2a). Nitrous acid deamination of the methyl ester methyl glycoside of neuraminic acid converts it mainly into the methyl ester methyl glycoside of the corresponding nonulosonic acid (see Fig. 1), and this can be analyzed[14] by g.l.c. (see Fig. 3). The methyl glycosides of simple hexoses remain intact in the deamination step.

Borohydride reduction. This is performed in order to convert the 2,5-anhydrohexoses into the corresponding alditols. 2,5-Anhydrohexitols are sufficiently stable as their trimethylsilyl derivatives for reliable, gas-chromatographic analysis[10,11,15] and their retention times are shorter than that of the methyl glycoside of D-mannose (see Fig. 3). On the SE-30 phase, 2,5-anhydrohexoses appear as peaks under those of the methyl glycosides of D-mannose and D-galactose.

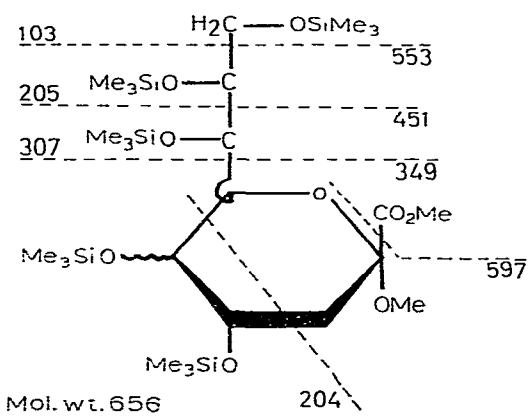
The reduction is performed, in water, with sodium borohydride[10] (see Experimental section), as reduction in the deamination reaction-mixture, which had been made alkaline by sodium hydroxide[11], gave wider variation in the amounts of 2,5-anhydrohexitols in g.l.c. analysis. Deaminated sialic acid cannot be analyzed by g.l.c. at this stage, due to the removal, under the alkaline conditions, of the methyl ester group at C-1. The reduction of substituted carboxyl groups has been reported[16,17],

and many attempts to achieve this have been made (in the presence of boric acid, and cation-exchange resins, and in anhydrous alcohols, as well as with alkaline-earth borohydride[18] in water, methanol, and oxolane), but all of them gave results unsatisfactory for quantitative work. The methyl group at the glycosidic carbon of methyl glycosides of simple hexoses and sialic acid prevents their reduction at this step.

Re-methanolysis. When the sample is subjected to a short re-methanolysis step after the borohydride reduction, the free carboxyl group of the nonulosonic acid is re-esterified. This step can be avoided if the sample is divided into two parts after deamination. One part is processed as already described for the analysis of simple hexoses and 2,5-anhydrohexitols, and the other is per(trimethylsilyl)ated directly after deamination for the analysis of deaminated sialic acid.

II. Structural analysis of deaminated amino sugar methyl glycosides and sialic acid methyl ester methyl glycoside

Nitrous acid deamination of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose, having equatorial amino groups, respectively produces[9] 2,5-anhydro-D-mannose and 2,5-anhydro-D-talose as the main products. For this work, the conditions of deamination described by Porter[10] were adopted, because they gave reproducible results, and had been applied for quantitative work. The mass spectrum of the trimethylsilyl derivative of the 2,5-anhydromannitol obtained by borodeuteride reduction is shown in Fig. 2a, with its major fragmentations, confirming the 2,5-anhydro structure. The molecular ions were not detected, but, from the ions at *m/z* 437 after borohydride reduction (438 after borodeuteride reduction; see Fig. 2a), 362 (363) and 272 (273), formed by the eliminations M — 15, M — 90, and M — (2 × 90), the molecular weight can be deduced. After elimination of C-1 or C-6, elimination of Me_3SiOH gives prominent ions at *m/z* 259 (259 and 260) and 169 (169 and 170). In the mass spectrum of 2,5-anhydromannitol, the ion at *m/z* 272 (273) has an in-



Scheme 1

tensity of 28% of the base peak, whereas, in the case of 2,5-anhydrotalitol, the intensity is only 3%.

Nitrous acid deamination of the methyl ester methyl glycoside of neuraminic acid was found to produce[14] one main peak in g.l.c. (peak 8 in Fig. 3) accompanied by several small peaks. The mass spectrum of the main peak and the major fragments of the supposed structure are shown in Fig. 2b and Scheme 1. The molecular ions were not detected, but ions at *m/z* 597 (600 for the trideuteriomethyl ester trideuteriomethyl glycoside) and *m/z* 566 (572), formed as M − 59 and M − 90, could be used for determination of the molecular weight. The intensity of the ion at *m/z* 204 was greater than that in the mass spectrum of *N*-acetylneuraminic acid[3,19], as part of the intensity originated from the fragment of the pyranoid ring containing C-4 and C-5. The ions at *m/z* 186 and 173, typical of the *N*-acetyl derivative, as well as those at *m/z* 144 and 131, typical of *N*-deacetylated neuraminic acid[20], were absent.

These data, supported by the results of the methylation analysis, indicated that the change in the size of the pyranoid ring is not the main reaction-path of neuraminic acid methyl ester methyl glycoside under nitrous acid deamination. The equatorial amino group on C-5 of neuraminic acid is simply replaced by a hydroxyl group, and, thus, the main product is methyl (methyl 3-deoxy-D-*glycero*-D-*galacto*-nonulosid)onate, a structure also suggested by Isemura and Schmid[21]. This conclusion is in agreement with the work of Lee and Schaffner[22], who found that deamination of the equatorially attached amino group of methyl 4-amino-4,6-dideoxy-α-D-mannopyranoside produced methyl 6-deoxy-α-D-mannopyranoside as the main product.

III. Gas chromatography

The analysis of standard monosaccharides by methanolysis, deamination, borohydride reduction, and remethanolysis is shown in Fig. 3. The relative retention

TABLE I

THE RETENTION TIMES[a] OF 2,5-ANHYDROHEXITOLS, AND DEAMINATED, *N*-DEACETYLATED, AND *N*-ACETYLATED SIALIC ACIDS, RELATIVE TO *myo*-INOSITOL, AS THEIR TRIMETHYLSILYL DERIVATIVES

Compound	*Stationary phase*		
	2.2% of SE-30	*1% of OV-101*	*3% of QF-1*
2,5-Anhydromannitol	0.430	0.492	0.163
2,5-Anhydrotalitol	0.545	0.506	0.195
Deaminated sialic acid	2.15	2.25	2.17
N-Deacetylated sialic acid	1.38	2.00	1.69
N-Acetylated sialic acid	2.91	4.64	7.69
myo-Inositol	**1.00**	**1.00**	**1.00**

[a]The analysis was performed isothermally at 140° for 2,5-anhydrohexitols, and at 190° for sialic acids.

TABLE II

RELATIVE, MOLAR-RESPONSE FACTORS IN G.L.C. AND G.L.C.–M.S. OF THE IONS AT *m/z* 204 AND 217 FOR 2,5-ANHYDROMANNITOL, 2,5-ANHYDROTALITOL, THE METHYL GLYCOSIDES OF L-FUCOSE, D-MANNOSE, D-GALACTOSE, AND D-GLUCOSE, AND THE METHYL ESTER METHYL GLYCOSIDE OF DEAMINATED SIALIC ACID AFTER RE-METHANOLYSIS, AS TRIMETHYLSILYL DERIVATIVES

Carbohydrate	*Peak number*[a]	*Response factor*			
		G.l.c.		*G.l.c.–m.s.*	
				m/z *204*	m/z *217*
L-Fucose	1	0.291	0.728	0.359	0.253
2,5-Anhydromannitol	2	0.264	0.661	—	0.824
2,5-Anhydrotalitol	3	0.125	0.313	—	0.797
D-Mannose	4	0.756	1.89	1.59	1.52
D-Galactose	5	0.400	1.000	1.000	1.000
D-Glucose	6	0.568	1.42	1.31	1.29
myo-Inositol	7	1.000	2.50	0.272	3.38
Deaminated sialic acid	8	0.148	0.370	0.015	0.342

[a]Numbers correspond to the peaks in Figs. 3, 4, and 5.

times of deaminated aminohexitols and neuraminic acid derivatives are given in Table I.

Methanolysis of each compound sugar gives a mixture whose chromatogram contains multiple peaks, because of anomerization and ring isomerization. For this reason, close attention was paid to the ratios of peak areas of different anomers and isomers; these ratios remained constant if the conditions were not changed. Therefore, only the areas of the largest peaks of each monosaccharide were used for calculations. The peaks selected for quantitation are numbered in Fig. 3 and in Table II, where the ratios of the peak areas to those of *myo*-inositol and D-galactose are given.

The detector responses given by the major deamination products showed a linear dependence on the concentration of amino sugars and sialic acid. The same was true for simple methyl hexosides[8]. The molar-response values relative to the internal standard were calculated from line slopes, and are given in Table II. The coefficients of variations (standard deviation expressed as per cent of the mean concentration) were below 10% for methyl glycosides of simple hexoses, and below 15% for 2,5-anhydrohexitols, deaminated sialic acid, and *myo*-inositol. For these calculations, the methyl glycoside of D-galactose was used as the internal standard.

The lower, molar-response values of 2,5-anhydrohexitols and deaminated sialic acid, relative to those of hexoses, are caused by the tendency of the deamination reaction to afford more than one product[9]. Under optimal conditions, the deamination of methyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride, with subsequent reduction of the product by buffered borohydride, has been shown to give 2,5-anhydro-D-mannitol in 59% yield[23].

Monosaccharide analysis of purified glycopeptide fractions could be readily performed by this method. When applied to a crude, protein fraction of rat brain,

the presence of unknown, background peaks made the analysis difficult, especially when packed g.l.c. columns were used. Therefore, the use of capillary columns, or g.l.c.–m.s., for the analysis of less-pure samples was found advisable.

IV. Combined g.l.c.–m.s.

When g.l.c.–m.s. is used, the monosaccharides are separated from each other by g.l.c., and then introduced into the mass spectrometer, where they are broken up into fragments typical of the native molecule. These structure-related fragments are monitored by mass fragmentography (selected-ion monitoring, s.i.m.). The method affords a specific way of quantitation, because impurities caused by unrelated structures are mostly avoided. The use of homologous, internal standards labelled with stable isotopes is also made possible. S.i.m. is a sensitive method making quantitation at the picomole level possible[3,4].

An essential benefit of the deamination reaction for g.l.c.–m.s. is the formation, from monosaccharides, of five- or six-membered ring-structures having many similarities in mass spectrometry. Common, intense ions, for all monosaccharides, at *m/z* 204 and 217, corresponding to ions at *m/z* 206 and 220 in the case of hexoses-d_7, are seen (see Table III and Figs. 2 and 4). The ion at *m/z* 204 represents, for the most part, the fragment containing C-2–C-3 and C-3–C-4 of the pyranoid ring. No deuterium labels introduced at C-1 or C-6 appear in this fragment. The absence of *m/z* 204, and the abundance of *m/z* 217, are typical of the mass spectra of Me_3Si derivatives of furanosides[2,4], and are characteristic in the mass spectra of 2,5-anhydrohexitols (see Fig. 2a). For sialic acid, the intensities of *m/z* 204 and 217 are greater in the mass spectrum of the deaminated than of the *N*-acetylated structure, because the former is trimethylsilylated at O-5 (see Fig. 2b and Scheme 1). The fragment having *m/z* 217 can be formed through different pathways, and has a relatively high

TABLE III

THE INTENSITIES OF THE IONS USED FOR SELECTED-ION MONITORING IN THE MASS SPECTRA OF THE TRIMETHYLSILYLATED MONOSACCHARIDES, GIVEN AS PERCENTAGE OF THE BASE-PEAK INTENSITY

Carbohydrate	*Peak number*[a]	m/z *value*			
		204	*206*	*217*	*220*
L-Fucose	1	100	9	19	—
2,5-Anhydromannitol	2	1	1	44	1
2,5-Anhydrotalitol	3	2	1	75	4
D-Mannose	4	100	9	25	1
D-Galactose	5	100	10	27	1
D-Glucose	6	100	5	27	1
myo-Inositol	7	19	2	70	1
Deaminated sialic acid	8	45	7	55	2
D-Galactose-d_7	5d	—	100	—	23

[a]Numbers refer to the corresponding peaks in Figs. 4 and 5.

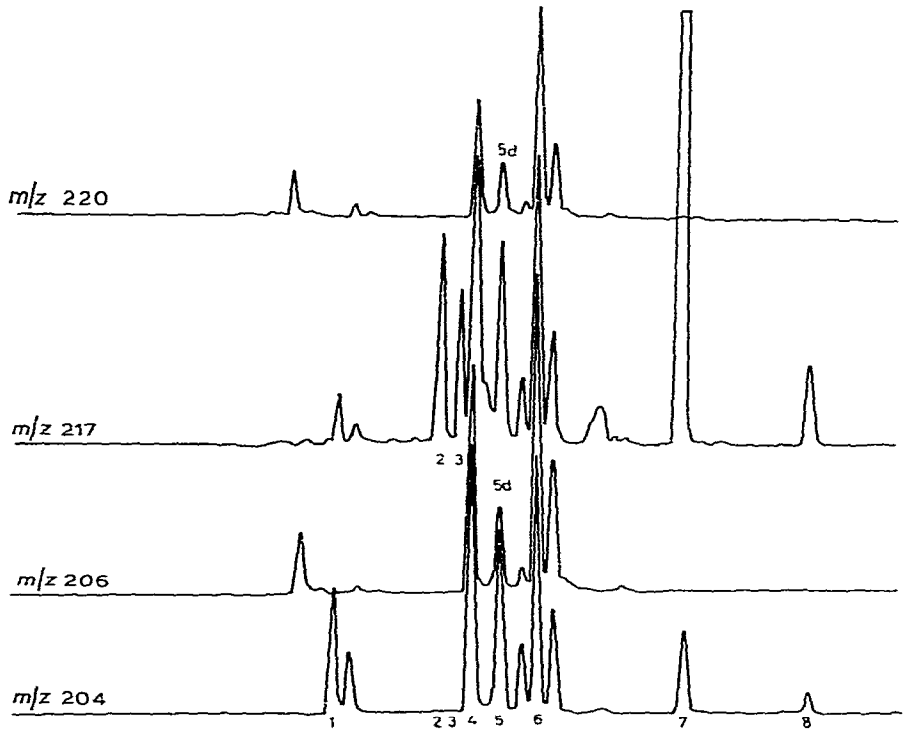


Fig. 4. A mass fragmentogram of a standard, sugar mixture, with *myo*-inositol and deuterated, algal-sugar mixture as internal standards. (The g.l.c.–m.s. conditions and peak numbers were as in Fig. 3 and Table II.)

intensity in the mass spectra of hexofuranosides, hexopyranosides[24], 2,5-anhydrohexitols, and deaminated sialic acid (see Fig. 2). The intensities of these fragments for different monosaccharides are given in Table III.

Besides *myo*-inositol, heptadeuterio-labelled monosaccharides of deuterated, algal sugar-mixture were also used as internal standards in mass fragmentography. The mixture contains considerable proportions of D-mannose, D-galactose, and D-glucose, and was found suitable for quantitation of these monosaccharides. All of their mass spectra had ions of great intensity at *m/z* 206 and 220 (see Table III). Heptadeuterio-labelled 2-acetamido-2-deoxy-D-hexoses would be useful internal standards in the analysis of amino sugars, but they were not available. The ions at *m/z* 204, 206, 217, and 220 were chosen for selected ion-monitoring.

When fragments at *m/z* 204 and 217 were recorded, the fragmentograms of a standard, sugar mixture were very similar to gas–liquid chromatograms (see Fig. 1 and 4). All peaks detected in g.l.c. appear in the fragmentograms at *m/z* 217, and they are numbered as in Table II. In the fragmentogram of *m/z* 204, the peaks of 2,5-anhydrohexitols were missing, as well as those of the furanosides of simple hexoses.

For calculation of the results, the molar amounts of different monosaccharides in the sample are first calculated relative to D-galactose, which is known to be a

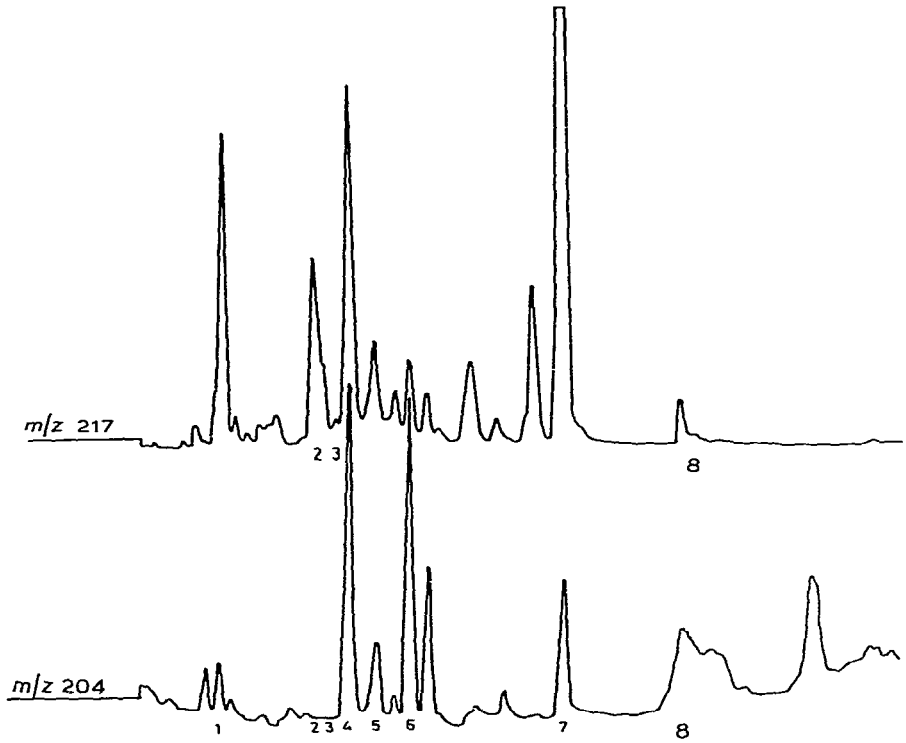


Fig. 5. A mass fragmentogram of the lipid-free, protein fraction of rat brain. (The g.l.c.–m.s. conditions and peak numbers were as in Fig. 3 and Table II.)

component of both *O*- and *N*-glycosylically linked glycoconjugates. The absolute amounts are obtained from the ratio of the D-galactose peak-area to that of D-galactose-d_7, after subtraction of the peak intensities caused by the nondeuterated fragments (the isotope peaks of *m/z* 204 and 217) under the deuterated fragments (*m/z* 206 and 220). The precision of mass fragmentography appeared to be the same as that of g.l.c., which emphasizes that the main error is involved in preparing the samples, and not in the determinations.

V. Application to biological material

A dry, lipid-free, protein fraction (500 μg) of rat brain was studied by this method. In gas–liquid chromatograms, an increased background made accurate analysis impossible, but the fragmentograms of the ions at *m/z* 204 and 217 were almost free from extra peaks (see Fig. 5). The disturbing peaks in the fragmentogram at *m/z* 204 overlapped with those of D-glucose and sialic acid, and, in the fragmentogram at *m/z* 217, with those of L-fucose. In these instances, quantitation was made on the basis of the fragmentogram having the smallest peak, also taking into consideration the characteristic pattern of peaks. The analysis was performed on the SE-30 phase entirely, and no column change was needed. The sample contained

L-fucose (9.4 nmol), D-mannose (17 nmol), D-galactose (9.6 nmol), D-glucose (13 nmol), 2-acetamido-2-deoxy-D-galactose (2.4 nmol), 2-acetamido-2-deoxy-D-glucose (16 nmol), and sialic acid (8.8 nmol) per mg of dry, lipid-free, protein residue. The results are in good agreement with those obtained from purified glycopeptides by different methods[12], considering the difference in the quality of the material analyzed and the precision of this method. The analysis could be performed on a 50-μg (dry weight) sample of protein. The analysis of the crude, protein sample showed the applicability of g.l.c.–m.s. in the quantitation of monosaccharides, especially in the case of small, less-pure samples.

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