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Gas chromatographic–mass spectrometric separation and characterization of methyl trimethylsilyl monosaccharides obtained from naturally occurring glycosides and carbohydrates

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ABSTRACT

Gas chromatographic–mass spectrometric (GC–MS) analysis of different methyl trimethylsilyl derivatives of aldohexoses, 6-deoxyaldohexoses and aldopentoses obtained after hydrolysis and trimethylsilylation of permethylated natural glycosides (*e.g.*, cardiac glycosides and saponins) is shown to be a reliable alternative method to the commonly applied analysis via partially methylated alditol acetates. GC and MS reference data for 25 monosaccharide derivatives are given, which allow the determination of the linkages in sugar chains of glycosides and polysaccharides. Mass spectra are characterized by fragmentation patterns, permitting conclusions even if reference substances or data are not available.

INTRODUCTION

In addition to other methods (*e.g.*, NMR techniques) gas chromatography–mass spectrometry (GC–MS) still remains an important method for the determination of the sugar moieties and their specific linkages in naturally occurring glycosides and carbohydrates. Normally the alditol acetates, obtained by hydrolysis of the permethylated compounds, reduction and acetylation, are analysed [1,2]. The preparation of the trimethylsilyl (TMS) derivatives of the hydrolysed products of permethylation so far is uncommon, although this method offers certain advantages. Direct trimethylsilylation without reduction results in two representative GC peaks corresponding to both anomeric sugar configurations with a constant relation of the peak areas [3,4]. Therefore, this method allows a more reliable identification of the particular monosaccharides.

This paper describes the GC–MS characterization of 25 TMS derivatives of monosaccharides after hydrolysis of various permethylated glycosides and oligosaccharides. The resulting reference data and the interpretation of the GC and MS data of the methyl TMS aldoses are given.

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EXPERIMENTAL

Methylation [5]

Approximately 2 mg of glycoside or carbohydrate are dried over phosphorus pentoxide and dissolved in 1 ml of dimethyl sulphoxide (DMSO), then 750 μl of methylsulphinyl carbanion are slowly added and stirred under nitrogen for 1 h at ≤30°C. (Preparation of methylsulphinyl carbanion [6]: 4.2 g of potassium *tert.*-butylate are dissolved in 12.5 ml of DMSO under nitrogen while stirring; the solution turns light yellow and stiffens when stored in the cool; for use, warm to 60°C). Then the solution is frozen in an ice-bath [sodium chloride–ice (1:3), −20°C] and 500 μl of iodomethane are slowly added. After thawing, the solution is stirred for 75 min under nitrogen and then diluted with 10 ml water. The solution is extracted five times with 10 ml of chloroform, the organic layer is washed three times with 10 ml water and dried with sodium sulphate and the chloroform is removed under reduced pressure [7].

Hydrolysis

A 0.5–1.0-mg amount of permethylated glycoside is heated with 0.3 ml of Kiliani mixture [8] (3.5 ml of concentrated acetic acid + 1.0 ml of concentrated hydrochloric acid + 5.5 ml of water) at 100°C for 2 h. The solution is neutralized on a Dowex 44 (OH^-) column (10 × 1 cm I.D.) with water and the eluate is dried with 2-butanone–1-propanol (1:1) under reduced pressure at ≤40°C [3,4].

Trimethylsilylation

The sugar derivatives are dissolved in dry pyridine (100 μl per 0.2 mg of sugar). To 100 μl of pyridine, 10 μl of hexamethyldisilazane and 10 μl trimethylchlorsilane are added [9] and 1 μl of this suspension is injected into the GC–MS system. The solution remains stable for several days (desiccator, 4°C).

GC–MS apparatus and conditions

A Shimadzu QP-1000 fused-silica capillary column (50 m × 0.25 mm I.D.) coated with SE-54-CB, film thickness 0.45 μm, was used, the split (1:10) being opened 0.5 min after injection, with a helium flow-rate of 2.5 ml/min, temperature programming from 100 to 250°C at 3°C/min, injection temperature 250°C, interface temperature 250°C, ion source temperature 170°C, ionization energy 20 eV, vacuum 3×10^{-6} Torr and mass range m/z 40–600 in 2 s.

Reagents

Potassium *tert.*-butylate, DMSO (distilled at 190°C with exclusion of water and stored over 4-nm molecular sieve under a nitrogen atmosphere), iodomethane (CH_3I) (distilled at 44°C and stored under nitrogen in the dark) (all of analytical-reagent grade from Merck, Darmstadt, Germany), trimethylchlorosilane and hexamethyldisilazane (Pierce, Rockford, IL, USA), β-D-allose, D-(+)-mannose (Sigma, St. Louis, MO, USA), D-(+)-glucose, D-(+)-galactose, L-(+)-arabinose, L-(−)-xylose, L-(+)-rhamnose (Merck), hyperoside, quercetrin dihydrate, α-sophorose monohydrate, gentiobiose (Roth, Karlsruhe, Germany), 1-(−)-fucose, cellobiose (Schuchardt, Munich, Germany) were used.

The glycosides were isolated in this institute and their structures were determined by fast atom bombardment MS and NMR spectroscopy: convalloside [10], neo-convalloside [10], glucoalliside [11], sarmentogenin-3-β-O-(β-D-6′-deoxyallopyranosido-4′-β-D-xylopyranosido-4″-α-L-rhamnopyranoside) [12], sarmentogenin-3-β-O-(β-D-6′-deoxyallopyranosido-4′-β-D-xylopyranosido-3″-β-D-apiofuranoside) [12], scilliphäosidin-3-O-(α-L-rhamnopyranosido-4′-D-glucopyranosido-3″-β-D-glucopyranoside) [13], 5α-4,5-dihydroscillirosidin-3-O-(α-L-6′-deoxyglucopyranosido-4′-β-D-glucopyranosido-4″-β-D-glucopyranoside) [14], 16β-O-acetylscillarenin-3-O-(β-D-glucopyranosido-4′-β-D-glucopyranoside) [15], scillarenin-3-O-(β-D-glucopyranosido-4′-β-D-glucopyranoside) [15], cyclamin [16], isocyclamin [16], giganteasaponin-1 [17] and -4 [18] and canadensissaponin A and B [19].

RESULTS AND DISCUSSION

After hydrolysis of the previously permethylated glycosides or carbohydrates, 25 different derivatives of the aldoses were trimethylsilylated. It was possible to separate all derivatives with high efficiency by GC using a specially adapted temperature programme. Identical monosaccharide derivatives obtained from different substances always showed identical retention times. Standard conditions during hydrolysis and trimethylsilylation resulted in constant ratios of the peak areas of the corresponding anomers. Therefore, the ratio may be used as additional tool for identification of a specific monosaccharide (Table I). As an example, the total ion current chromatogram of aldose derivatives obtained from giganteasaponin 4 [18] is given in Fig. 1.

Optimization of the MS parameters was necessary to obtain a high response for the most relevant fragment ions: reduction of the ion source temperature to 170°C and consequent use of a 20-eV ionization energy [7] caused a significant depression of the ions at *m/z* 73 and 147 corresponding to the TMS functions, whereas the fragment ions essential for interpretation increased at the same time.

The MS data showed high reproducibility and therefore results regarding the identity of each sugar obtained by GC were confirmed. As differently substituted sugar monomers were represented by distinct MS fragmentations, information about the substitution of sugars that were not examined could also be obtained.

The sample preparation scheme (permethylation, hydrolysis and trimethylsilylation) results in the substitution of the glycosidic hydroxyl group with TMS in each instance. Therefore, the interpretation of the mass spectra is simple and we recommend a step-by-step procedure to define the residues at C-1 to C-4 for all aldoses (hexoses, 6-deoxyhexoses and pentoses) and at C-6 for hexoses. For this purpose, it is sufficient to compare the intensities of some specific fragment series (J, F/G, H and K series, following the nomenclature of Kochetkov and Chizhov [20] and Petersson and Samuelson [21,22]) (Table I). The most intensive fragment in each of these series represents the substituent of a particular carbon atom.

The stepwise interpretation is performed as follows:

C-1: $-$O-TMS in all instances.

C-3: if the ion at *m/z* at 133 is more intense than that at *m/z* 191 (J series), C-3 must be substituted by $-$O-CH_3, whereas if the ion at *m/z* 191 is more intense than that of *m/z* 133, C-3 must be substituted by $-$O-TMS.

C-2: the fragments at *m/z* 88, 146 or 204 (H series) must be compared. A

TABLE I

GC AND MS DATA FOR ALL THE SUGARS ANALYSED

The C atoms with −O-TMS groups are listed; all others are $-O-CH_3$ substituted. FV (%) = ratios of the two GC peak areas of one sugar. Abundances of 0% represent traces less than 0.49%; dashes indicate not detected. Values are the averages of all measurements for each sugar. The fragment series (J, H, F/G and K) are assigned according to refs. 20–22.

Assignment		GC data		Abundance of MS fragments as % of the base peak														
Position of −O-TMS	Sugar	FV (%)	Retention time (min:s)	J series			H series			F/G series			K series					
													Hexoses			6-Deoxy hexoses		Pentoses
				m/z 75	*m/z* 133	*m/z* 191	*m/z* 88	*m/z* 146	*m/z* 204	*m/z* 101	*m/z* 159	*m/z* 217	*m/z* 102	*m/z* 160	*m/z* 218	*m/z* 72	*m/z* 130	*m/z* 58	*m/z* 116
1	Allose	100	32:32	10	100	–	71	1	–	59	4	–	5	2	–	3	–	–	1
		20	34:10	10	100	–	61	2	–	46	20	–	8	–	–	6	–	–	1
	Glucose	74	31:52	11	100	0	69	2	0	42	6	0	11	1	0	3	1	0	1
		100	32:06	9	100	0	72	2	0	32	13	1	8	2	1	2	1	0	1
	Mannose	100	32:54	8	100	0	70	2	–	30	3	0	4	0	0	1	0	0	0
		10	33:24	5	100	–	47	6	2	22	2	1	3	–	–	1	–	–	–
	Galactose	100	32:46	5	100	0	58	1	0	34	4	0	6	0	–	4	0	2	2
		78	33:26	6	100	–	51	0	–	32	5	0	6	1	0	–	2	1	1
	Rhamnose	100	25:50	14	79	0	100	11	–	14	2	0	3	1	–	14	3	1	2
		10	26:10	11	72	0	100	6	0	55	3	0	1	1	–	17	1	1	1
	Fucose	86	24:34	11	53	–	100	6	0	23	1	–	2	1	–	19	1	2	1
		100	25:08	14	62	–	100	3	–	29	2	–	2	1	–	22	3	4	3
	Arabinose	100	26:44	0	100	1	26	1	1	72	1	1	–	–	–	3	0	6	2
		52	27:26	0	100	2	36	21	2	45	8	7	4	2	2	1	–	7	–
	Xylose	100	24:28	5	100	1	60	3	1	53	2	2	3	1	1	–	1	7	2
		83	24:40	9	100	2	68	2	–	40	3	3	5	1	0	1	1	9	2
	Apiose	–	25:46	100	7	–	–	–	–	20	5	–	–	–	–	3	–	–	–
1,2	Glucose	100	36:24	7	100	2	25	84	1	14	77	0	1	12	1	7	3	0	1
		60	37:30	2	100	2	2	68	3	2	88	14	–	15	3	8	3	0	1
	Rhamnose	100	27:20	–	100	2	17	59	4	0	22	1	0	3	1	7	1	0	1

		45	28:36	0	100	3	11	46	2	0	22	2	2	0	4	6	1	1	1
1,3	Glucose	100	36:40	5	1	82	–	100	–	8	3	3	3	1	1	1	–	–	1
		64	37:38	7	0	100	–	91	–	15	3	1	3	–	1	1	–	–	1
	Xylose	100	29:08	11	6	81	1	100	–	21	7	2	2	1	1	1	1	0	1
		68	30:18	8	4	75	–	100	–	22	6	6	1	1	1	–	3	2	3
1,4	Glucose	100	36:50	3	99	–	74	4	1	8	60	4	2	23	3	1	2	0	2
		76	37:06	1	100	1	50	4	3	7	44	5	1	17	3	1	1	0	1
	6-Deoxy-	25	31:30	6	100	6	95	2	–	8	68	–	2	5	–	–	13	–	3
	allose	100	31:50	1	100	3	80	8	–	4	61	–	–	6	1	–	24	–	–
	6-Deoxy-	100	30:34	2	84	6	100	21	4	4	19	4	0	12	1	0	26	1	2
	glucose	85	31:02	1	100	5	84	14	2	6	29	2	0	4	1	–	27	1	1
	Rhamnose	100	30:50	5	16	6	100	19	2	5	16	6	2	5	1	1	23	–	1
		65	31:10	8	22	5	87	7	3	8	22	5	–	4	6	–	22	–	–
	Xylose	100	29:36	–	68	–	50	10	–	12	100	7	4	96	2	11	1	2	10
		92	29:58	5	83	5	68	5	2	15	100	6	1	81	6	23	4	–	17
1,6	Glucose	100	35:22	8	100	2	58	3	1	25	8	2	3	6	0	2	2	0	1
		90	36:12	7	100	9	57	2	21	24	8	2	3	6	–	0	2	0	1
1,2,3	Glucose	100	41:38	1	14	79	2	11	46	3	18	1	1	2	4	0	0	0	0
		93	43:52	0	11	75	3	5	68	3	15	14	1	1	20	1	2	1	2
	6-Deoxy-	38	34:58	3	6	49	1	31	100	1	7	4	1	2	1	3	2	1	1
	glucose	100	37:54	2	6	100	0	18	97	1	7	3	1	1	1	2	0	–	–
	Arabinose	85	34:36	0	10	65	0	61	100	–	42	5	1	9	1	0	0	1	5
		100	36:50	3	7	64	1	33	100	–	36	6	0	7	1	0	0	–	1
1,2,4	Arabinose	100	33:02	2	20	1	4	31	2	3	6	100	0	0	20	0	1	0	3
		94	33:18	3	20	2	2	36	1	3	10	96	0	1	80	1	1	1	4
1,2,6	Glucose	75	38:46	12	88	5	3	100	4	3	95	6	1	24	1	1	2	0	3
		100	41:20	5	59	1	6	100	4	2	57	0	1	14	3	1	1	1	4
1,3,4	Rhamnose	100	35:00	2	7	87	0	100	68	1	14	3	–	2	1	2	11	0	1
		15	37:02	1	2	60	–	100	10	–	10	2	–	1	1	1	10	–	0

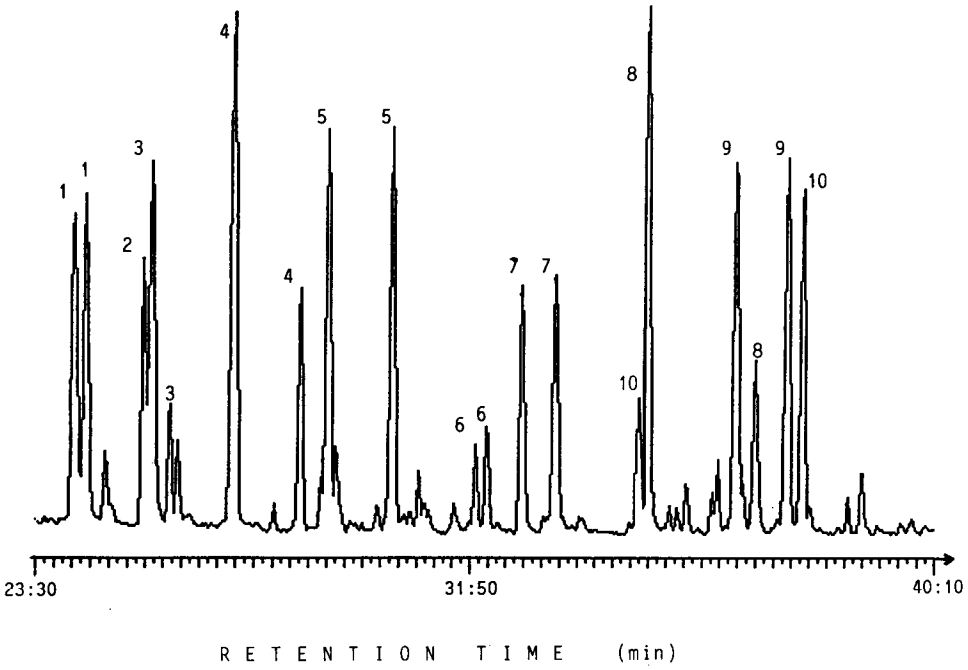

Fig. 1. Total ion chromatogram from 23 min 30 s to 40 min 10 s of partially methylated trimethylsilylated monosaccharides obtained from giganteasaponin 4 [18] on SE-54-CB with temperature programming from 100 to 250°C at 3°C/min. Identified peaks: 1 = 2,3,4,-trimethyl-1-TMS-xylose; 2 = 2,3,3'-trimethyl-1-TMS apiose; 3 = 2,3,4-trimethyl-1-TMS-rhamnose; 4 = 3,4-dimethyl-1,2-TMS-rhamnose; 5 = 2,4-dimethyl-1,3-TMS-xylose; 6 = 2,3,4,6-tetramethyl-1-TMS-glucose; 7 = 2,3,4,6-tetramethyl-1-TMS-galactose; 8 = 2-methyl-1,3,4-TMS-rhamnose; 9 = 2,4,6-trimethyl-1,3-TMS-glucose; 10 = 4-methyl-1,2,3-TMS-6-deoxyglucose.

dominant ion at *m/z* 88 represents $-O-CH_3$ and at *m/z* 204 $-O$-TMS groups at C-2 and C-3, respectively. A most abundant ion at *m/z* 146 shows one $-O-CH_3$ and one $-O$-TMS at these positions; considering the apparent substituent at C-3, one can determine the substituent at C-2.

C-4: the fragments at *m/z* 101, 159 or 217 (F/G series) must be compared. A dominant ion at *m/z* 101 represents $-O-CH_3$ and at *m/z* 217 $-O$-TMS groups on C-2 and C-4, respectively. A most abundant ion at *m/z* 159 shows one $-O-CH_3$ and one $-O$-TMS at these positions; considering the apparent substituent at C-2, one can determine the substituent at C-4.

C-6: depending on the substitution pattern of C-4 and C-6 of hexoses (two $-O-CH_3$ or one $-O-CH_3$ + one $-O$-TMS or two $-O$-TMS groups), the most intensive ion of a series of three must be considered (K series): *m/z* 102 or 160 or 218. As the substituent at C-4 is known, one can determine the substituent at C-6.

With 6-deoxyhexoses the K series (*m/z* 72 for C-4 $-O-CH_3$ or *m/z* 130 for C-4 $-O$-TMS) gives further confirmation of the substituent at C-4.

In addition, all results were confirmed by analysis of permethylated (the dominant J series peak is at *m/z* 75) and pertrimethylsilylated sugars which showed analogous fragmentation patterns [7].

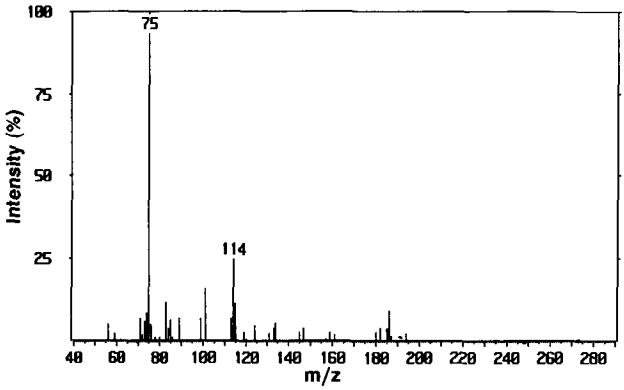

Fig. 2. Mass spectrum of the artifact representing 2,3,3′-trimethyl-1-TMS-apiofuranoside after GC. For details, see text.

It should be stated that the given procedure is only suitable for pyranosides, but we found all sugars investigated to be pyranoses; furanosides are recognized according to the literature [20–22] by a base peak in the F/G series and very weak H series ions (maximum *ca.* 10%). The occasional occurrence of genuine partially methylated sugars in natural products must also be taken into consideration: a parallel analysis without prior methylation (only hydrolysis and pertrimethylsilylation) gives the respective information [3,4]. The differentiation of hexoses, 6-deoxyhexoses and pentoses by comparison of the intensities of the seven K series ions (for pentoses *m/z* 58 if C-4 $-O-CH_3$ or *m/z* 116 if C-4 $-O$-TMS) was not possible because five sugars (3-methyl-1,2,4-TMS-arabinose, 4-methyl-1,2,3-TMS-arabinose, 4,6-dimethyl-1,2,3-TMS-glucose, 2,4-dimethyl-1,3-TMS-xylose and 2,3-dimethyl-1,4-TMS-xylose) gave contradictory results.

As an exceptional case the 2,3,3′-O-trimethyl-1-O-TMS-apiofuranoside should be mentioned; this derivative is destroyed by acid hydrolysis, but always appears as the same reproducible artifact represented by a GC peak at 25 min 46 s. The corresponding mass spectrum shows fragments at *m/z* 75 (base peak), 114 (23%), 101 (20%), 115 (6%) and 83 (5%) (Fig. 2).

However, it should be pointed out that the MS results must be evaluated very carefully. The few exceptions found during these investigations show that the use of MS data as the only method for identification is not always reliable. On the other hand, it is definitely possible to determine sugars and their substitution patterns as long as reference data for retention times, peak area relationships and MS fragmentations are available.

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