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Review

Characterization of neutral sugars and uronic acids after methanolysis and trimethylsilylation for recognition of plant gums

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Abstract

The main standard neutral sugars and uronic acids that occur as components of plant gums were methanolysed and silylated for study by gas chromatography–electron impact and chemical ionization mass spectrometry (GC–EI-MS and GC–CI-MS). The 25 TMSi methylglycosides, components of the chromatograms obtained were studied and identified by their mass spectra and/or comparison with the corresponding standards. In addition, an unusual uronic acid (4-O-methylglucuronic acid) and the lactone forms of glucuronic acid are reported. A classification of the ions in both EI-MS and CI-MS which allows differentiation between sugar classes and their tautomeric forms is given. The sample preparation method and the results of the above identification were applied to the analysis of some plant gums and a seventeenth century ink sample.

Contents

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1. Introduction

Plant gums consist of complex, highly branched polysaccharides composed of neutral sugars and hexuronic acid monomers. These natural products were used in the past as binding agents in different mixtures, e.g., as constituent parts of metallo-gallic inks [1], Egyptian ointments for mummification [2] and underlayers of paints and painted sculptures [3]. They now find numerous applications as thickening agent or emulsion stabilizers in the agroalimentary, cosmetic and pharmaceutical industries.

Because of their diversity and chemical complexity, the identification of plant gums requires a series of complex analytical procedures. However, a reliable classification may be achieved on the basis of the monosaccharide composition after stoichiometric cleavage of glycosidic bonds. Possible specific or total depolymerization reactions have been reported previously [4]. A major difficulty has always involved the decomposition of the liberated monomers when drastic conditions are necessary to ensure the breakdown of the more resistant glycosidic bonds. With respect to that particular difficulty, methanolysis may be preferable to the classical acid hydrolysis since it gives higher recoveries of both neutral sugars and uronic acids [4–7].

As recently reviewed [8], many chromatographic methods have been devised for the identification and quantification of carbohydrates. Owing to high sensitivity combined with an ability to achieve efficient separations of complex mixtures, gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) have gained general acceptance for the analysis of trimethylsilylated (TMSi) methanolysates of glycoproteins [5–6,9] and commercial gums [10].

Numerous GC and/or MS studies on TMSi carbohydrates have been reported. The fragmentations produced in electron impact (EI) of neutral sugar derivatives (including glucose, methyl glucosides and methyl galactosides) [11] and pertrimethylsilylated hexuronic acids and their lactones [12] were elucidated using high-resolution measurements and/or deuterium labelling. The assignment of structural features from the resulting mass spectra was also discussed. Further, GC–MS analyses of neutral sugars and galacturonic acid produced by methanolysis of pectins have been published [13]. Particular attention was paid to the differences between the EI spectra of non-isomeric methyl glycosides. However, the EI mass spectra suffered from a lack of resolution between the chromatographic peaks of certain ring tautomers.

In addition to the well established EI-MS, chemical ionization (CI)MS has proved to be a valuable technique, especially with regard to the differentiation of isomeric sugars. Meaningful results have been obtained by GC coupled with ammonia chemical ionization mass spectrometry (NH_3 CI-MS) for the analysis of pertrimethylsilylated derivatives of glucose, mannose and galactose [14]. Except for a study concerned with methyl xylosides, methyl glucosides and the 6-methyl ester 1-methyl glycoside of 4-O-methylglucuronic acid [15], NH_3 CI-MS data related to TMSi methyl glycosides are scarce.

In this investigation, extensive GC–EI-MS and

GC–NH_3 CI-MS data were obtained for TMSi derivatives of standard neutral sugars and uronic acids commonly produced by the methanolysis of plant gums. The potential of this approach is illustrated by its application to the characterization of three plant gums and organic constituents of a seventeenth century ink sample.

2. Experimental

2.1. Materials

Materials and reagents were all of analytical-reagent grade. Standard carbohydrates (free sugars and methyl glycosides) and plant gums were obtained from Aldrich (Milwaukee, WI, USA), Merck (Darmstadt, Germany), Fluka (Buchs, Switzerland) and Sigma (St. Louis, MO, USA). The trimethylsilylation reagent SYLON-HTP, consisting of pyridine, hexamethyldisilazane and trimethylchlorosilane (9:3:1, v/v/v), was purchased from Supelco (Bellefonte, PA, USA).

2.2. Sample preparation

Methanolysis and trimethylsilylation were carried out according to the procedures of Ha and Thomas [10] and Sweeley et al. [16], respectively. Standard carbohydrates or plant gums samples (typically 1.5 mg) were taken up in 0.5 ml of methanolic HCl solution prepared by adding acetyl chloride (0.4 ml) to 15 ml of methanol. Methanolysis was conducted at 80°C for 24 h. Thereafter, methanol and HCl were removed using a nitrogen stream and without prior neutralization since the acid was largely "consumed" at this stage of the methanolysis procedure [9]. An excess of the trimethylsilylation agent (0.5 ml) was added to the dried material. The solutions were then heated at 80°C for 2 h. Subsequently, the derivatized samples were evaporated using rotary evaporation at 50–60° and the residue was immediately dissolved in 0.5 ml of hexane. GC–MS analysis was performed with 1 μl of this solution.

2.3. Gas chromatography–mass spectrometry

The GC–MS system consisted of a Series 3400 gas chromatograph (Varian, Walnut Creek, CA, USA) interfaced by direct coupling to an INCOS 50 quadrupole mass spectrometer (Finnigan, San Jose, CA, USA). The gas chromatograph was equipped with a 30 m × 0.25 mm I.D. fused-silica column coated with a 0.25-μm film of DB-5 poly(5% phenyl–95% methylsiloxane) (J & W Scientific, Folsom, CA, USA). The carrier gas was helium at a flow-rate of 1.3 ml/min (measured at 40°C). The injector and transfer line temperatures were set to 300 and 250°C, respectively. Two different sets of chromatographic conditions were used. In the case of standard monosaccharides, injection was performed in the split mode with a splitting ratio of 50:1 and the oven temperature was raised from 150 to 220°C at 2°C/min. In the case of gum and ink samples, splitless injection (splitless time 30 s; splitting ratio 50:1) was followed by the oven temperature programme 40–130°C at 9°C/min, 130–290°C at 2°C/min and 290°C for 10 min.

EI and NH_3 CI mass spectra were measured in the total ion monitoring mode. The operating conditions for EI-MS were source temperature 100°C, filament emission current 750 μA, ionizing voltage 70 eV and scan range from m/z 29 to 650 with a period of 1.7 s. The other operating parameters were those set by the instrument's automatic calibration routine. Typical NH_3 CI-MS conditions were as follows: ion source temperature 120°C, filament emission current 750 μA, ionizing voltage 110 eV and scan range from m/z 150 to 650 with a period of 1.4 s. To ensure reproducible pressure conditions, the analyser forepressure (0.04 Torr) was monitored and the reagent gas flow-rate (1.2 ml/min) was kept constant by means of a mass flow controller. Under these conditions, the NH_4^+:$N_2H_7^+$ and $N_2H_7^+$:$N_3H_{10}^+$ ratios were about 5.6 and 55.5, respectively.

3. Results and discussion

3.1. Gas chromatography of standard monosaccharides

The study of reference monosaccharides involved a series of neutral sugars and uronic acids commonly found in most plant gums. The chromatographic patterns of the products obtained when the methanolysis–trimethylsilylation procedure was applied to the individual sugars are shown in Fig. 1.

Methanolysis of a given sugar leads to the formation of several products owing to the well known anomerization and ring isomerization processes. The number of glycoside peaks (which ranges between two and four), their retention times and relative proportions are characteristic of each monosaccharide. Obviously, this multiple peak pattern facilitates identification, although a rather complex chromatogram will result. The high efficiency provided by capillary columns generally permits satisfactory separations to be achieved, even when mixtures of sugars are analysed. All the tautomeric forms are well resolved except for arabinose, galactose and fucose derivatives (Fig. 1). The separations are greatly improved with the temperature programming used for the characterization of plant gums (see Fig. 5). However, mass spectra monitored across the third GC peak of fucose revealed the presence of a minor furanoside form, not resolved from the prominent pyranoside. Successful separation of these ring form isomers appears impossible to achieve on the capillary column used, despite various tentative temperature programmes.

3.1.1. Identification of pentose and hexose anomers

Although spectra are useful for attributing furanosides and pyranosides structures, they are of limited use for the identification of α- and β-anomers [11]. Thus, assignments of α- and β-pyranoside forms are made by injection of authentic methyl glycosides (methyl β-D-arabinopyranoside, methyl β-D-xylopyranoside, methyl α-D-mannopyranoside, methyl β-D-galactopyranoside and methyl α-D-glucopyranoside) as TMSi derivatives, whereas α- and β-furanoside forms are assigned according to previously reported kinetics of glycoside formation [17,18]. Except for arabinose derivatives, comparison of results obtained for these five sugars with a previous chromatographic study of per-trimethylsilylated glycosides [19,20] reveals a close agreement with regard to the elution order (α before β) observed within a given anomeric pair, and with a chemically similar stationary phase.

However, free sugars and the corresponding methyl glycosides show noticeable differences in their anomeric equilibrium compositions. Indeed, α-pyranose forms of most free sugars in aqueous or pyridine solutions are known to be thermodynamically less stable (and thus less abundant) than β-anomers [21]. Nevertheless, we can see in Fig. 1 that α-pyranosides are predominant in all sugars except arabinose. Such an increase or inversion of the α:β ratios can be explained by a strong anomeric effect in methanolic HCl [21]. If the $^4C_1$ chair conformation is assumed [22], the stability is enhanced when the C-1 substituent is axially attached, i.e., in the case of the α-anomers. In contrast, arabinose, whose pyranoside forms are known to favour the $^1C_4$ conformation [17], is expected to yield predominantly the β-anomer, as observed.

3.1.2. Identification of 6-deoxyhexose anomers

Methyl glycosides of rhamnose (6-deoxymannose) and fucose (6-deoxygalactose) were not commercially available as pure anomers. NMR investigations of equilibrium composition in aqueous solutions [21] have shown that 6-deoxyaldohexoses and the corresponding aldohexoses afford an almost identical anomeric distribution. The same regularity should be assumed to be valid for the methyl glycosides. This assumption, combined with the similarities between the chromatographic patterns of hexoses and their 6-deoxy analogues, allows us to attribute α- and β-forms of methyl fucosides and methyl rhamnosides as shown in Fig. 1.

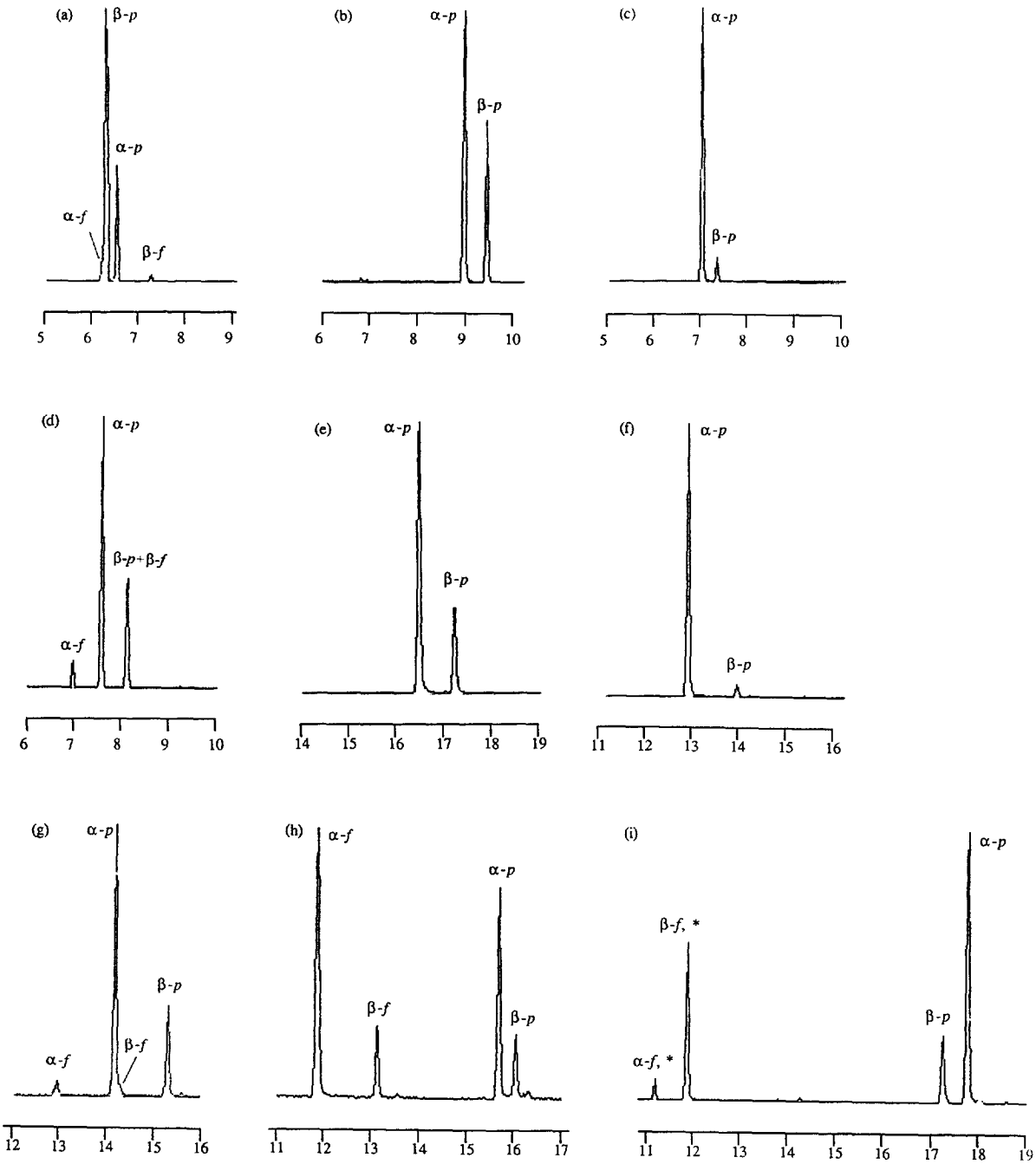

Fig. 1. Total ion current chromatograms (recorded in EI mode) of reaction mixtures obtained from methanolysis and trimethylsilylation of standard monosaccharides: (a) arabinose; (b) xylose; (c) rhamnose; (d) fucose; (e) glucose; (f) mannose; (g) galactose; (h) galacturonic acid; (i) glucuronic acid (lactone forms are asterisked). Elution time is in minutes.

3.1.3. Particularity of uronic acids

Both investigated uronic acids yield four products on methanolysis. The third and fourth peaks on the chromatogram of glucuronic acid correspond to the expected TMSi α- and β-1-methyl glycoside 6-methyl esters. The remaining peaks can be identified as methyl glycosides of glucofuranuro-6,3-lactone by comparison with reported EI mass spectra of pertrimethylsilylated hexuronic acid and lactones [12]. This identification was further confirmed by injection of an authentic sample of glucurono-6,3-lactone. Owing to their characteristic mass spectra, the occurrence of these methanolysis products (obtained in reproducible proportions) is very helpful for ensuring the presence of glucuronic acid in an unknown sample. Although many hexuronic acids are known to lactonize to some extent in acidic media, including methanolic HCl solutions, the configuration of galacturonic acid precludes lactonization [23]. Hence methanolysis of this acid yields four components corresponding to methyl α- and methyl β-furanosides and pyranosides of the methyl uronate.

Assignments of the anomeric configurations are possible by comparison with HPLC data on the methanolysis of glucuronic and galacturonic acid [23].

3.2. Electron impact mass spectrometry

A great number of EI-MS data regarding permethylated glycosides [24] and pertrimethylsilylated glycosides [11,12] have been published. MS data from TMSi methyl glycosides are more scarce but, as demonstrated by De Jongh et al. [11] for the glucose derivatives, mass spectra of TMSi methylglycosides and of pertrimethylsilylated glycosides present evident analogies. The fragmentations are the same except that some peaks are shifted by 58 mass units, which can be accounted for by the difference between CH_3O and $(CH_3)_3SiO$ at C-1 (glycosidic carbon). For instance, the peak at m/z 191 $[(CH_3)_3SiOCH=O^+Si(CH_3)_3]$, very intense in the mass spectra of pertrimethylsilylated glycosides, is largely (ca. 80%) shifted to m/z 133 $[CH_3OCH=O^+Si(CH_3)_3]$ in the mass spectra of TMSi methyl glycosides. The peak at m/z 191 is then very helpful for the rapid identification (mass fragmentogram) of pertrimethylsilylated glycosides that can be present in small amounts (less than 1%) in the derivative mixtures. Methyl glycosides may be effectively partially converted into free sugars at the end of methanolysis, in the methanol removal step, even when carefully prepared anhydrous methanolic HCl is used [25].

GC–MS analyses of TMSi methyl glycosides from sugars contained in pectins have been reported by Petrzika and Linow [13]. These sugars were galacturonic acid and all the neutral sugars concerned in the present study. In their fundamental paper, mass spectral data are discussed in detail with consideration of fragmentation patterns. They used a packed column and the resulting power of separation was lower than that obtained in the present work with a capillary column. They described sixteen different TMSi methyl glycosides, whereas for the same set of compounds, and under similar methanolysis conditions, we separated 23 tautomers. Consequently, Petrzika and Linow [13] reported some mass spectra as resulting from pure tautomeric form, whereas they result from superposition of two different mass spectra.

Our results are given in two manners: characteristic mass spectra are presented in bar graph form (Fig. 2) and selected ions from all the mass spectra are listed in Tables 1 and 2.

The structures and classification of ions obtained from TMSi methyl pyranosides and furanosides are shown in Fig. 3 using, as in Table 1, the nomenclature originally created by Kochetkov and Chizhov [24] and adapted by Petrzika and Linow [13]. This nomenclature, initially developed for permethylated glycosides, can be applied to TMSi methyl glycosides with some modifications. For these compounds, the glycosidic carbon atom C-1 is substituted by OCH_3 when carbon atoms C-2 to C-4 are substituted by OTMSi and, thus, two different ions separated by 58 mass units may be observable for a specific fragment series. For the fragment series B, J, F and H, these two ions are denoted a and b.

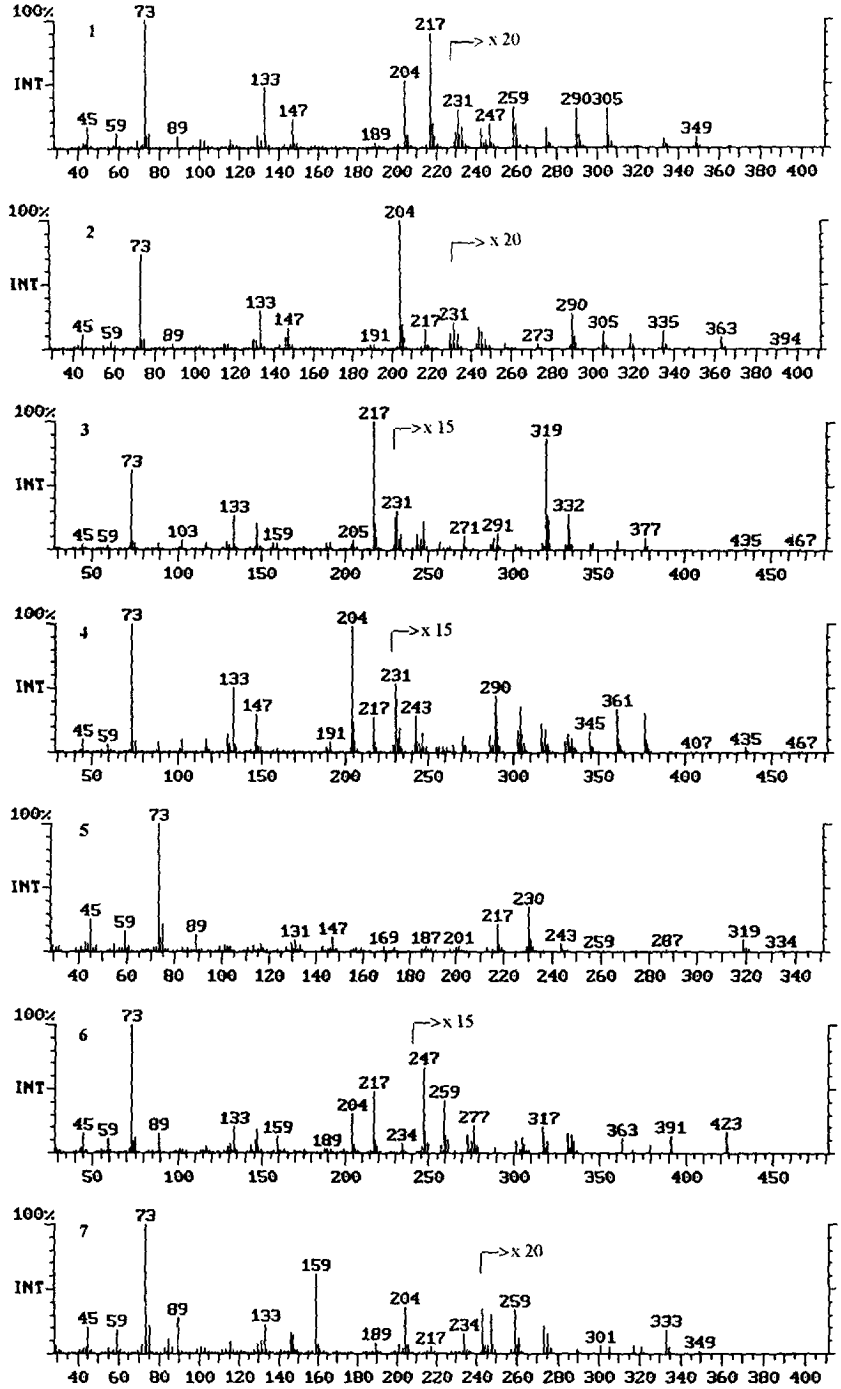

Fig. 2. EI mass spectra of TMSi methyl glycosides: (1) methyl β-arabinopyranoside; (2) methyl α-rhamnopyranoside; (3) methyl α-galactofuranoside; (4) methyl α-glucopyranoside; (5) methyl β-glucofuranosidurono-6,3-lactone; (6) methyl (methyl α-glucopyranosid)uronate; (7) methyl (methyl α,β-4-O-methyl glucopyranosid)uronate (first isomer eluted).

Table 1
EI mass spectra of TMSi methyl glycosides

Methyl glycoside[a]	Structure[b]	M_r	*m/z* (relative intensity, %)											
			A_1	A_2	A_3	B_{2a}	B_{2b}	B_{2c}	J_{1a}	J_{1b}	$F1_a/G_1$	F_{1b}	C_{2a}	C_{2b}
Arabinose 1	α-*f*	380	349 (0.2)	259 (0.9)	169 (0.5)	305	247 (0.6)	335 (0.2)	191 (1.0)	133 (10.4)	217 (100.0)	159 (1.5)	231 (0.9)	230 (3.3)
Arabinose 2	β-*p*	380 (0.01)	349 (0.4)	259 (1.6)	169 (1.2)	305 (1.5)	247 (0.9)	335 (0.1)	191 (1.6)	133 (46.5)	217 (90.2)	159 (1.6)	231 (1.5)	230 (0.6)
Arabinose 3	α-*p*	380	349 (0.3)	259 (1.8)	169 (1.2)	305 (1.2)	247 (0.7)	335 (0.1)	191 (1.6)	133 (44.1)	217 (99.8)	159 (1.4)	231 (1.3)	230 (0.6)
Arabinose 4	β-*f*	380	349	259 (0.5)	169 (0.6)	305	247 (0.9)	335	191 (1.0)	133 (10.1)	217 (100.0)	159 (1.6)	231 (1.0)	230 (3.0)
Xylose 1	α-*p*	380	349 (0.8)	259 (2.0)	169 (1.3)	305 (1.1)	247 (2.1)	335	191 (2.5)	133 (32.7)	217 (68.0)	159 (2.2)	231 (1.2)	230 (1.1)
Xylose 2	β-*p*	380	349	259 (2.0)	169 (1.5)	305 (0.9)	247 (2.4)	335	191 (1.8)	133 (28.9)	217 (60.2)	159 (1.5)	231 (1.2)	230 (0.9)
Rhamnose 1	α-*p*	394 (0.02)	363 (0.5)	273 (0.2)	183 (0.3)	305 (0.7)	247 (0.4)	335 (0.7)	191 (3.5)	133 (29.0)	217 (14.8)	159 (1.4)	245 (0.7)	244 (0.8)
Rhamnose 2	β-*p*	394	363	273 (0.2)	183 (0.5)	305 (0.7)	247 (0.3)	335 (0.3)	191 (2.4)	133 (27.1)	217 (16.0)	159 (0.8)	245 (0.4)	244 (0.1)
Fucose 1	α-*f*	394	363	273 (0.5)	183 (0.4)	305	247 (2.9)	335 (0.6)	191 (3.0)	133 (20.0)	217 (100.0)	159 (5.1)	245 (0.5)	244 (0.6)
Fucose 2	α-*p*	394	363 (0.9)	273 (0.6)	183 (0.6)	305 (4.3)	247 (1.0)	335 (0.4)	191 (3.9)	133 (42.0)	217 (19.4)	159 (1.8)	245 (2.3)	244 (0.1)
Fucose 3	β-*f* + β-*p*	394	363	273 (0.2)	183 (0.5)	305 (1.7)	247 (0.5)	335 (0.2)	191 (2.8)	133 (38.7)	217 (18.0)	159 (1.5)	245 (0.9)	244 (0.1)
Mannose 1	α-*p*	482	451	361 (1.2)	271 (0.5)	305 (1.8)	247 (0.8)	335 (1.0)	191 (10.1)	133 (52.9)	217 (29.2)	159 (3.0)	333 (0.6)	332 (1.3)
Mannose 2	β-*p*	482	451	361 (0.5)	271 (0.6	305 (1.7)	247 (0.5)	335 (0.3)	191 (5.3)	133 (43.0)	217 (22.0)	159 (1.9)	333 (0.3)	332 (0.5)
Galactose 1	α-*f*	482	451	361 (0.4)	271 (0.7)	305 (0.2)	247 (1.4)	335 (0.2)	191 (4.4)	133 (25.9)	217 (100.0)	159 (4.3)	333 (0.6)	332 (1.8)
Galactose 2	α-*p*	482	451	361 (1.5)	271 (0.4)	305 (2.2)	247 (1.0)	335 (0.6)	191 (5.2)	133 (39.4)	217 (25.8)	159 (1.9)	333 (0.5)	332 (0.4)
Galactose 3	β-*f*	482	451	361 (0.2)	271 (0.4)	305	247 (1.5)	335 (0.1)	191 (4.4)	133 (24.8)	217 (100.0)	159(4.5)	333 (0.4)	332 (1.9)
Galactose 4	β-*p*	482	451	361 (0.3)	271 (0.2)	305 (1.5)	247 (0.4)	335 (0.4)	191 (4.5)	133 (39.4)	217 (21.7)	159 (1.4)	333 (0.3)	332 (0.2)
Glucose 1	α-*p*	482	451	361 (1.7)	271 (0.6)	305 (1.8)	247 (0.7)	335 (0.5)	191 (7.7)	133 (49.9)	217 (27.0)	159 (2.6)	333 (0.4)	332 (0.7)
Glucose 2	β-*p*	482	451	361 (0.4)	271 (0.5)	305 (1.5)	247 (0.6)	335 (0.3)	191 (4.9)	133 (41.4)	217 (22.7)	159 (1.7)	333 (0.3)	332 (0.4)
Galacturonic acid 1	α-*f*	438	407 (0.3)	317	227 (1.7)	305 (0.2)	247 (4.2)	335 (0.2)	191 (1.0)	133 (18.3)	217 (97.6)	159 (7.3)	289 (0.8)	288 (1.3)
Galacturonic acid 2	β-*f*	438	407	317	227 (1.1)	305	247 (4.5)	335	191 (1.0)	133 (16.7)	217 (77.6)	159 (7.5)	289 (0.7)	288 (1.1)
Galacturonic acid 3	α-*p*	438	407 (0.5)	317 (1.0)	227 (0.8)	305 (1.0)	247 (4.3)	335 (0.4)	191 (2.4)	133 (18.2)	217 (52.7)	159 (9.5)	289 (0.2)	288
Galacturonic acid 4	β-*p*	438	407	317 (0.3)	227 (0.6)	305 (0.5)	247 (3.6)	335	191 (1.4)	133 (16.7)	217 (47.4)	159 (7.2)	289 (0.3)	288
Glucuronic acid 1	β-*p*	438	407	317 (1.3)	227	305	247 (4.3)	335	191 (1.7)	133 (17.6)	217 (48.1)	159 (10.3)	289	288
Glucuronic acid 2	α-*p*	438	407	317 (1.3)	227 (0.6)	305 (0.8)	247 (4.4)	335 (0.6)	191 (3.0)	133 (20.2)	217 (48.2)	159 (13.3)	289 (0.3)	288
4-O-Methylglucuronic acid 1	α,β-*p*	380	349 (0.1)	259 (1.7)	169 (1.6)	305[c] (0.2)	247 (1.5)	277 (0.2)	191[c] (0.6)	133 (21.9)	217[c] (4.2)	159 (61.6)	231 (0.5)	230 (0.2)
4-O-Methylglucuronic acid 2	α,β-*p*	380	349	259 (0.8)	169 (1.1)	305[c] (0.3)	247 (1.0)	277	191[c] (1.9)	133 (23.0)	217[c] (7.9)	159 (43.5)	231 (1.1)	230 (0.2)

Structure assignments of fragment ions (A–K) refer to the nomenclature of Kochetkov and Chizhov [24] as adapted by Petrzika and Linow [13].

[a] Tautomeric forms are numbered in their elution order.

[b] *p* = Pyranoside; *f* = furanoside.

[c] These ions are partially shifted 58 mass units lower owing to the methoxyl group on C-4.

Table 1 (*continued*)

m/z (relative intensity, %)											
C_{2c}	C_3	H_{1a}	H_{1b}	K_1	E_{1b}	$M^{\cdot+} - CH_3^{\cdot}$	$M^{\cdot+} - CH_3^{\cdot}$ −TMSIOH	$M^{\cdot+} - CH_3^{\cdot}$ −2 TMSIOH	$M^{\cdot+} - CH_3^{\cdot}$ $-CH_3OH$	$M^{\cdot+} - CH_3^{\cdot}$ $-CH_3OH$ −TMSIOH	$M^{\cdot+}$ − TMSIO$^{\cdot}$ −TMSIOH
319	141 (0.2)	204 (0.7)	146 (0.9)	116 (1.9)	277 (0.1)	365	275 (0.6)	185 (0.4)	333 (0.4)	243 (0.7)	201 (0.7)
319	141 (0.6)	204 (51.7)	146 (2.5)	116 (6.3)	277 (0.1)	365 (0.1)	275 (0.8)	185 (0.4)	333 (0.3)	243 (0.7)	201 (2.7)
319	141 (0.5)	204 (50.7)	146 (2.4)	116 (6.0)	277 (0.1)	365	275 (0.8)	185 (0.4)	333 (0.4)	243 (0.8)	201 (2.1)
319	141 (0.3)	204 (0.7)	146 (0.7)	116 (1.5)	277 (0.2)	365	275 (1.2)	185 (0.4)	333 (0.3)	243 (1.2)	201 (0.6)
319	141	204 (84.6)	146 (5.3)	116 (6.9)	277	365 (0.3)	275 (0.9)	185 (0.6)	333	243 (0.9)	201 (5.0)
319	141	204 (81.1)	146 (2.4)	116 (6.1)	277	365 (0.1)	275 (1.0)	185(0.7)	333 (0.5)	243 (0.9)	201 (3.3)
319 (0.6)	155 (0.6)	204 (100.0)	146 (8.6)	130 (7.8)	277	379	289 (0.1)	199 (0.2)	347	257 (0.2)	215 (1.5)
319 (0.3)	155 (0.6)	204 (100.0)	146 (2.4)	130 (4.9)	277	379	289 (0.4)	199 (0.3)	347 (0.1)	257 (0.3)	215 (0.8)
319 (1.0)	155 (0.4)	204 (2.6)	146 (2.4)	130 (1.0)	277 (0.3)	379	289 (0.6)	199 (0.9)	347 (0.1)	257 (0.6)	215 (0.6)
319 (1.5)	155 (1.3)	204 (100.0)	146 (3.8)	130 (8.9)	277	379	289 (0.4)	199	347 (0.2)	257 (0.3)	215 (1.1)
319 (0.5)	155 (1.0)	204 (88.6)	146 (1.3)	130 (6.1)	277	379	289 (0.5)	199 (0.4)	347 (0.2)	257 (0.4)	215 (0.8)
319 (1.1)	243 (1.3)	204 (100.0)	146 (8.3)	218 (7.4)	277	467 (0.1)	377 (0.8)	287 (0.8)	435 (0.1)	345 (0.5)	303 (0.3)
319 (0.9)	243 (1.1)	204 (96.6)	146 (2.8)	218 (5.5)	277	467	377 (1.2)	287 (0.8)	435	345 (0.6)	303 (0.1)
319 (5.8)	243 (0.8)	204 (4.0)	146 (1.8)	218 (20.0)	277	467	377 (0.6)	287 (0.3)	435 (0.1)	345 (0.2)	303 (0.1)
319 (0.7)	243 (1.8)	204 (100.0)	146 (2.7)	218 (6.2)	277	467	377 (0.6)	287 (0.3)	435 (0.1)	345 (0.3)	303 (0.4)
319 (4.8)	243 (0.6)	204 (5.2)	146 (1.6)	218 (18.2)	277 (0.1)	467	377 (0.9)	287 (0.5)	435	345 (0.2)	303 (0.2)
319 (0.4)	243 (1.3)	204 (97.6)	146 (1.1)	218 (4.8)	277	467	377 (0.8)	287 (0.3)	435	345 (0.3)	303 (0.3)
319 (0.9)	243 (1.4)	204 (98.8)	146 (7.5)	218 (7.2)	277	467	377 (1.5)	287 (0.6)	435 (0.2)	345 (0.8)	303 (0.9)
319 (0.5)	243 (1.1)	204 (98.9)	146 (3.2)	218 (5.7)	277	467	377 (2.0)	287 (0.6)	435 (0.1)	345 (0.7)	303 (0.6)
319 (1.5)	199 (0.4)	204 (1.0)	146 (4.3)	174 (0.4)	277 (5.0)	423 (2.0)	333 (0.7)	243 (1.1)	391 (0.5)	301 (0.7)	259
319 (1.7)	199 (0.5)	204 (0.3)	146 (3.7)	174 (0.3)	277 (3.7)	423	333 (1.3)	243 (1.8)	391 (0.6)	301 (1.3)	259 (0.3)
319 (0.7)	199 (3.2)	204 (57.8)	146 (5.3)	174 (0.7)	277 (0.5)	423 (1.4)	333 (0.7)	243 (0.6)	391 (0.7)	301 (0.2)	259 (2.0)
319	199 (2.0)	204 (51.0)	146 (3.2)	174	277	423	333 (0.5)	243	391	301 (0.3)	259 (1.7)
319	199 (1.6)	204 (31.3)	146 (8.3)	174 (0.4)	277 (0.4)	423 (0.5)	333 (1.4)	243	391	301	259 (2.0)
319 (0.6)	199 (2.5)	204 (30.1)	146 (10.2)	174 (0.9)	277 (1.4)	423 (1.1)	333 (0.9)	243 (0.5)	391 (0.9)	301 (0.6)	259 (2.7)
261 (0.6)	199 (1.3)	204[c] (35.4)	146 (16.3)	116 (8.4)	219 (1.8)	365	275 (0.7	185 (0.4)	333 (0.9)	243 (1.7)	201 (6.5)
261 (0.3)	199 (0.8)	204[c] (44.8)	146 (10.2)	116 (5.8)	219 (1.8)	365	275 (0.5)	185 (0.3)	333 (0.7)	243 (1.4)	201 (2.4)

(*Continued on p. 36*)

Table 1 (*continued*)

m/z (relative intensity, %)																										
73	89	101	103	115	116	117	129	131	143	147	157	189	203	207	215	221	230	231	233	234	260	261	278	290	321	331
49.9	3.6	1.5	6.8	1.0	1.9	1.7	3.5	2.8	1.2	7.0	0.5	2.8			2.4	0.5	3.3	0.9								
100.0	8.3	6.7	5.6	1.5	6.3	2.9	9.3	5.3	3.0	22.6	1.2	3.3	0.9	1.5	1.6	2.3	0.6	1.5	0.8		0.9	0.3		1.5		
100.0	8.0	6.3	6.3	1.4	6.0	2.7	7.1	5.2	2.6	19.5	1.0	3.9	1.5	1.6	1.4	2.2	0.6	1.3	0.9		0.5	0.3		1.5		
43.2	4.7	1.3	6.1	0.9	1.5	1.8	3.4	2.6	1.4	7.2	0.5	2.2			2.4	0.6	3.0	1.0			0.2					
100.0	6.6	6.7	5.9	1.8	6.9	3.6	10.8	6.3	2.4	27.2	1.1	3.6	3.5	3.6	1.8	4.0	1.1	1.2	1.3					1.9		
100.0	9.1	6.1	4.8	1.7	6.1	2.9	9.2	5.2	2.7	20.9	0.9	3.8	3.1	2.6	1.4	3.1	0.9	1.2	1.6					1.6		
74.2	3.2	2.0	2.7	3.2	2.6	4.1	5.3	6.4	2.2	16.3	0.8	2.9	1.5	1.4	1.5			1.0	0.6					1.3	0.1	
70.6	6.1	1.8	1.7	3.2	2.3	4.8	3.6	5.3	3.2	14.3	0.8	3.8	0.9	1.3	0.8			1.3	0.8					1.1		
75.6	4.7	5.1	3.7	1.6	4.3	38.1	5.1	7.1	2.7	11.4	1.4	11.6			0.6			2.9	0.9						0.1	
93.5	5.3	3.7	2.7	4.7	3.0	5.5	6.6	7.4	7.5	25.4	1.9	4.4	0.7	1.6	1.1			0.9	0.8		0.5			2.3	0.2	
100.0	8.7	3.7	2.6	4.4	2.9	8.6	5.1	6.1	5.8	20.7	1.3	6.1	0.6	1.2	0.8			0.8	0.9					1.5	0.1	
94.6	7.1	2.9	9.9	0.9	4.1	13.1	15.9	7.8	2.2	28.6	1.9	4.9	3.8	2.1	0.5	1.0		3.6	0.8			0.2		2.4	0.4	0.6
100.0	10.1	2.8	8.2	0.8	3.2	10.1	12.1	5.9	1.9	25.1	1.6	4.0	2.4	1.7		0.8		2.5	0.9					1.7	0.3	0.4
62.1	4.7	1.6	7.0	0.5	3.0	4.8	5.6	4.0	1.6	19.7	4.4	4.7	1.2	1.1		0.7	1.6	2.0	0.8						1.5	0.3
98.6	6.0	2.2	8.9	0.8	2.6	6.4	15.8	5.0	2.5	23.8	1.0	3.0	2.2	2.5		0.7		1.3	0.5		0.8	0.4		1.8	0.2	0.4
52.3	6.1	1.9	6.5	0.7	2.3	5.3	4.9	4.2	1.5	19.9	3.2	4.2	1.3	1.4		0.8	1.3	1.4	0.9						0.8	0.1
100.0	8.3	2.2	7.9	0.8	2.4	5.5	12.4	4.5	2.2	23.0	1.0	3.3	1.7	2.0		0.6		1.2	0.7			0.3		1.3	0.2	0.2
100.0	8.1	2.6	9.4	0.9	3.3	10.0	13.9	6.6	2.0	29.6	1.4	4.1	3.2	2.3		0.9		2.6	1.0			0.2		2.2	0.4	0.5
100.0	9.6	2.3	7.8	0.8	2.8	8.9	9.5	5.3	1.8	23.6	1.1	4.1	2.3	1.9		0.7		2.2	1.1			0.2		1.8	0.3	0.3
100.0	15.1	2.6	4.3	1.0	3.6	1.9	6.9	6.8	3.1	10.5	2.5	3.0	0.5		0.9	0.6	0.7	0.8	1.5	7.9		1.9	1.2	0.3	0.2	
100.0	18.3	3.0	5.4	1.2	3.5	2.1	7.1	6.1	2.9	9.4	3.0	2.2		0.5	1.1	0.5	1.0	1.3	1.5	6.7		3.7	1.3			
100.0	14.1	2.8	3.2	1.2	4.6	2.5	4.7	6.3	4.1	18.3	1.4	3.4	0.9	4.4	1.0	0.8		0.8	1.2	8.1	2.0	0.9			0.1	2.1
100.0	15.2	3.2	3.8	1.4	4.7	2.5	4.6	6.3	4.0	14.5	0.8	3.6	0.6	2.8	0.6			0.6	0.9	6.5	0.3					0.6
100.0	15.5	3.2	3.1	1.0	5.4	2.8	5.6	7.0	5.0	15.0		4.2		1.3	1.0				0.5	6.3						0.4
100.0	15.2	2.5	2.8	1.0	4.7	2.1	4.9	6.8	5.7	18.2	1.2	3.1	0.6	2.0	1.2	0.7		0.6	0.9	6.6	0.8	0.7	0.4			1.0
100.0	27.1	5.0	3.6	1.5	8.4	3.0	6.8	10.1	1.3	13.7	1.1	7.4	3.5	0.7	1.7		0.2	0.5	1.2	15.3	0.4	0.6			0.2	
100.0	18.2	3.4	4.1	1.3	5.8	4.2	6.6	7.0	1.2	11.5	0.8	5.9	4.5	0.9	1.0		0.2	1.1	1.0	9.0	0.1	0.2		0.5	0.1	

Table 2
EI mass spectral data for TMSi methyl glycosides of α-glucofuranuro-6,3-lactone (**1**) and β-glucofuranuro-6,3-lactone (**2**)

m/z	Relative intensity (%)		*m/z*	Relative intensity (%)	
	1	**2**		**1**	**2**
334		0.5	159	1.8	2.5
319	6.9	10.2	157	3.0	2.4
291		0.7	147	14.9	15.5
287	2.0	1.6	143	7.6	4.6
259	1.5	1.0	133	5.9	6.2
243	6.2	6.5	131	10.7	10.6
230	40.0	34.7	129	8.5	7.4
229	4.8	3.0	117	3.1	2.9
217	25.9	23.1	116	6.0	6.5
213	2.6	2.3	115	1.4	1.6
201	2.4	3.0	113	6.0	4.9
197	6.3	1.8	103	4.8	3.0
187	4.2	4.4	101	5.6	5.0
173	2.9	2.5	89	24.5	12.5
169	5.6	4.7	73	100.0	100.0

The mass spectra also contain additional fragmentation series due to losses of a methyl or trimethylsiloxy group from ion radicals (see Table 1). The ion B2c is also produced by loss of a methyl group from B_1 [11,13].

In the C series, C_{2a} corresponds to C_2 in the nomenclature of Kochetkov and Chizhov [24]. C_{2b} and C_{2c} are produced from C1: C_{2b} by loss of trimethylsilanol, C_{2c} by loss of the C-6 carbon substituent. Ions of the E series are formed by elimination of the side chain: E_{1a} (absent in the spectra) by cleavage of the C-5–C-6 bond for the pyranose forms, E_{1b} by cleavage of the C-4–C-5 bond for the furanose forms (in the E_{1b} column some values corresponding to pyranose forms cannot be attributed to the E_{1b} structure, despite the mass number).

Except for 4-O-methylglucuronic acid and glucurono-6,3-lactone and without consideration of the ring size, anomeric configuration or other stereochemical features such as axial or equatorial configuration, the sugars studied differ only in the nature of the substituent at C-5. This C-5 substituent corresponds to a hydrogen for the pentoses, to a CH_3 group for the deoxyhexoses, to a CH_2OTMS group for the hexoses and to a $COOCH_3$ group for the uronic acids.

For ions retaining the C-5 substituent, the *m/z* value varies with the class of sugar, as it can be seen for ions of series A, C (except C_{2c}) and K and series due to losses of a methyl or trimethylsiloxy group from molecular ion.

For ions losing the C-5 substituent, the *m/z* value is the same for all the sugars, as can be seen for C_{2c} ion and for ions of series B, J, F, H and E.

Some other ions do not contain any carbon of the ring. For instance, ions at *m/z* 73 $[Si^+(CH_3)_3]$ and *m/z* 147 $[(CH_3)_3SiOSi^+(CH_3)_2]$ are present in the mass spectra of all TMSi derivatives of carbohydrates and, in the mass spectra of TMSi methylglycosides, the ion at *m/z* 89 $[CH_3OSi^+(CH_3)_2]$ is to ca. 75% an ion analogous to *m/z* 147.

3.2.1. Determination of ring size (see Fig. 2, spectra 3 and 4)

The intensity of the ion at *m/z* 204 (H_{1a}) is closely related to ring size. Production of ions at *m/z* 204 requires a circulation of electrons within the ring, which is favoured by a six-atom cyclic structure [12,24]. For the pyranose forms the relative intensity of the ion at *m/z* 204 (relative to the intensity of the base peak) varies between 30% and 100%. The maximum relative intensity

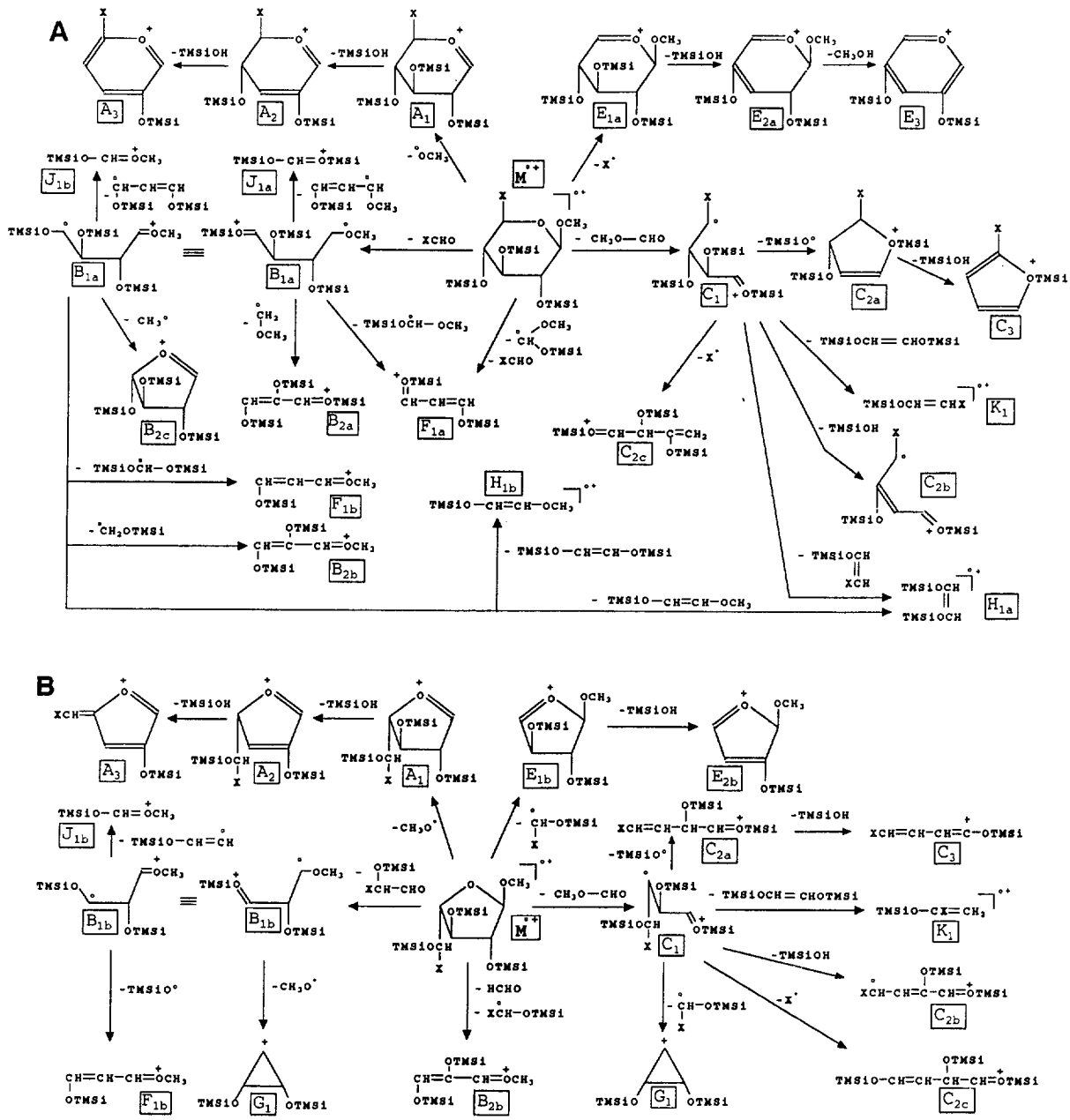

Fig. 3. Structures of ions obtained by EI-MS of (A) TMSi methyl glycopyranosides and (B) TMSi methyl glycofuranosides. The ion classification refers to the nomenclature of Kochetkov and Chizhov [24] as adapted by Petrzika and Linow [13] and applies to pentoses (X = H), hexoses (X = CH_2OTMSi), 6-deoxyhexoses (X = CH_3), and methyl uronates (X = CO_2CH_3) (adapted from Ref. [13]).

of the ion at m/z 204 observed for furanose forms is 5.2% (galactose 3).

Several isomeric ions correspond to the peak at m/z 217 [11,24]. The major one is the F_{1a} ion for the pyranose forms and the G_1 ion for the furanose forms. The ion at m/z 217 is generally very intense for the furanose forms (base peak for the neutral sugars). The difference in the

relative intensities of the ion at m/z 217 for the furanose and the pyranose forms of the same compound is greatly dependent on the compound.

There still remain a number of characteristic features which differentiate the mass spectra of furanosides from those of pyranosides. In the mass spectra of furanosides, ion at m/z 305 (B_{2a}) is almost absent. In contrast, C_{2b}-type ions have an increased intensity. For the neutral sugar furanose forms, the absence of an ion at m/z 290 and a decreased intensity of the ion at m/z 133 (J_{1b}) are additional characteristics.

The relatively high intensity of the ion at m/z 319 (C_{2c}) appears to be characteristic of the hexofuranosides. For methyl arabinoside derivatives, differences in the mass spectra of furanosides and pyranosides are also pronounced for the ions at m/z 146 (H_{1b}), 116 (K_1) and 201 ($M^{\cdot+} - TMSiO^{\cdot} - TMSiOH$).

3.2.2. Differentiation of sugar classes

3.2.2.1. General. The molecular ion is generally absent in the mass spectra of the studied sugar derivatives. It is observed at trace levels for arabinose 2 and rhamnose 1 (Table 1) and with higher relative intensity (0.5%) for the glucurono-6,3-lactone derivatives (Table 2).

Ions in the high-mass range are also generally of weak intensity and observed only for relatively high concentrations of sample.

Few ions are absolutely specific for the different sugar classes. The differentiation is generally established from a set of different ions.

3.2.2.2. Characterization of pentoses (see Fig. 2, spectrum 1). Pentoses are characterized by the presence in the mass spectra of ions at m/z 365 ($M^{\cdot+} - CH_3^{\cdot}$), 349 ($A_1$: $M^{\cdot+} - CH_3O^{\cdot}$), 333 ($M^{\cdot+} - CH_3^{\cdot} - CH_3OH$), 275 ($M^{\cdot+} - CH_3^{\cdot} -$ TMSiOH) and 259 (A_2: $M^{\cdot+} - CH_3O^{\cdot} -$ TMSiOH). These ions are also present, with different intensities, in the mass spectra of the 4-O-methylglucuronic acid derivatives (same molecular mass) but additional characteristic peaks help to avoid confusion. The ion at m/z 259 ($M^{\cdot+}$-TMSiO$^{\cdot}$ – TMSiOH) is also present in the mass spectra of galacturonic and glucuronic acid derivatives.

Differentiation between TMSi derivatives of methyl arabinosides and methyl xylosides can be easily established by using the m/z 204/217 ratio: the ratio is <1 for methyl arabinosides and >1 for methyl xylosides.

3.2.2.3. Characterization of hexoses (see Fig. 2, spectra 3 and 4). Hexoses are characterized by the presence of ions at m/z 467 ($M^{\cdot+} - CH_3^{\cdot}$), 451 ($A_1$: $M^{\cdot+} - CH_3O^{\cdot}$), 435 ($M^{\cdot+} - CH_3^{\cdot} - CH_3OH$) and especially 377 ($M^{\cdot+} - CH_3^{\cdot} -$ TMSiOH), 361 (A_2: $M^{\cdot+} - CH_3O -$ TMSiOH) and 345 ($M^{\cdot+} - CH_3^{\cdot} - CH_3OH$-TMSiOH), the first three peaks being observed only for relatively high concentrations of compound.

In the mass spectrum of glucose 1 (see Fig. 2, spectrum 4), an ion of very low intensity present at m/z 407 corresponds to the loss of $CH_3^{\cdot}$ from C_1 ion ($M^{\cdot+} - CH_3OCHO$) [11]. A subsequent loss of TMSiOH gives ion at m/z 317. These types of ions (C_1, $C_1 - CH_3^{\cdot}$ and $C_1 - CH_3^{\cdot} -$ TMSiOH) are not mentioned in Table 1 because they are not detected for most of the sugars studied and because of possible confusion with isobaric ions for pentoses, deoxyhexoses and 4-O-methylglucuronic acid derivatives. The ion at m/z 407 is also observed in the mass spectra of mannose 1 and galactose 2. The ion at m/z 317 is present with relatively high intensity in the mass spectra of pyranose forms.

The three hexoses are not easily differentiated from the mass spectra of pyranose forms. However, TMSi derivatives of methyl galactosides are identified from mass spectra of the furanose forms.

3.2.2.4. Characterization of deoxyhexoses (see Fig. 2, spectrum 2). Deoxyhexoses are characterized by the presence in the mass spectra of ions at m/z 394 ($M^{\cdot+}$), 363 (A_1: $M^{\cdot+} - CH_3O^{\cdot}$), 347 ($M^{\cdot+} - CH_3^{\cdot} - CH_3OH$), 273 ($A_2$: $M^{\cdot+} - CH_3O^{\cdot} -$ TMSiOH) and 257 ($M^{\cdot+} - CH_3^{\cdot} - CH_3OH -$ TMSiOH). Ions at m/z 363 and 273

are also present in the mass spectra of uronic acids (see Fig. 2, spectrum 6).

The major methyl glycosides of rhamnose and fucose (rhamnose 1 and fucose 2) may be differentiated from ions at m/z 245 (C_{2a}: $M^{\cdot+} - CH_3OCHO - TMSiO$) and 244 ($C_{2b}$: $M^{\cdot+} - CH_3OCHO - TMSiOH$): the m/z 244/245 ratio is >1 for TMSi methyl rhamnosides and <1 for TMSi methyl fucosides. Fucose is also identified from mass spectrum of its TMSi methyl α-furanoside.

3.2.2.5. Characterization of galacturonic and glucuronic acids derivatives (see Fig. 2, spectrum 6). The TMSi derivatives of these uronic acids are easily characterized by the presence in the mass spectra of ions at m/z 423 ($M^{\cdot+} - CH_3^{\cdot}$), 391 ($M^{\cdot+} - CH_3^{\cdot} - CH_3OH$), 317 ($A_2$: $M^{\cdot+} - CH_3O^{\cdot} - TMSiOH$), 247 ($B_{2b}$: $M^{\cdot+} - CHOCOOCH_3 - TMSiOCH_2^{\cdot}$) and 234.

The formation of the odd-electron ion at m/z 234, very characteristic of TMSi derivatives of uronic acids, has been partially explained by a complex McLafferty-type rearrangement of a trimethylsilyl group to the carboxyl group [12].

In the mass spectrum of glucuronic acid 2 (see Fig. 2, spectrum 6), the ion at m/z 363 corresponds, as for glucose 1, to the loss of $CH_3^{\cdot}$ from C_1 ion ($M^{\cdot+} - CH_3OCHO$) [12]. A subsequent loss of TMSiOH gives the ion at m/z 273. These types of ions are not mentioned in Table 1. The two ions are also present in the mass spectrum of galacturonic acid 4 [m/z 363 (0.5%) and 273 (1.4%)]. These m/z values correspond to A_1 and A_2 in the mass spectra of deoxyhexoses.

The ion at m/z 407 (A_1) is present in the mass spectra of galacturonic acid 1 and 3. This m/z value corresponds to $C_1 - CH_3^{\cdot}$ in the mass spectra of hexoses.

The ion at m/z 159 (F_{1b}), present in the mass spectra of all the studied sugars, is of higher intensity (7–13% of the base peak) than in the mass spectra of neutral sugars. The ion at m/z 277 (E_{1b}: $M^{\cdot+} - TMSiOCH^{\cdot}COOCH_3$) is especially intense for the furanose forms.

Pyranose forms of the two uronic acids may be differentiated by using the m/z 204/217 ratio: the ratio is >1 (1.1) for TMSi derivatives of galacturonic acid and <1 (0.6) for TMSi derivatives of glucuronic acid.

3.2.2.6. Characterization of 4-O-methylglucuronic acid derivatives (see Fig. 2, spectrum 7). Only two pyranose forms are observed for this uronic acid. The presence of a methoxy group at C-4 precludes the formation of furanose forms.

TMSi derivatives of 4-O-methyl glucuronic acid and pentoses have the same molecular mass. Isobaric ions are then found in the mass spectra of the two sugar classes and especially the series of characteristic ions: m/z 349 (A_1), 333 ($M^{\cdot+} - CH_3^{\cdot} - CH_3OH$), 275 ($M^{\cdot+} - CH_3^{\cdot} - TMSiOH$) and 259 ($A_2$).

In contrast, the ion at m/z 234 (15% of the base peak for the major isomer) is characteristic of uronic acids. The McLafferty-type rearrangement previously mentioned for the formation of m/z 234 [12] cannot be directly applied to 4-O-methylglucuronic acid derivatives since it requires the presence of a TMSiO group at C-4.

The fragmentations of 4-O-methylglucuronic acid derivatives are the same as the fragmentations of the two other uronic acids except that the C-4-containing ions are shifted by 58 mass units (difference between CH_3O and TMSiO substituent). This feature is especially observed for ions F_{1a} which generally contain C-2, C-3 and C-4: m/z 217 is almost completely shifted to m/z 159 and an anomalously high value is observed in the F_{1b} column. The high relative intensity of m/z 159 (61.6% for the major isomer) and the very low relative intensity of m/z 217 (4.2% for the major isomer) are then characteristic of the 4-O-methylglucuronic acid derivatives.

The same feature is observed, but less pronounced, for ion H_{1a} (m/z 204) partially shifted to H_{1b} (m/z 146). H_{1a} may effectively contain C-2, C-3 or C-3, C-4. The shift of B_{2a} to B_{2b} and J_{1a} to J_{1b} should be also expected, but the observed values are not conclusive.

3.2.2.7. Characterization of glucurono-6,3 lactone derivatives (see Fig. 2, spectrum 5). Selected ions are listed in Table 2. The mass spectra of

TMSi methyl glucurono-6,3-lactones are mainly characterized by the presence of an intense odd-electron at m/z 230, the major ion following the m/z 73 ion. This rearrangement ion is also present with the same m/z value in the spectra of persilylated lactones [12]. For the major isomer, the molecular ion is observed at m/z 334 (0.5% of the base peak).

The $(M^{\cdot+} - CH_3^{\cdot})$ ion at m/z 319 is especially intense (7% and 10% of the base peak). However, C_{2c} ions of relatively high intensity are also present at the same m/z value in the mass spectra of hexofuranosides derivatives.

The ion at m/z 287 $(M^{\cdot+} - CH_3^{\cdot} - CH_3OH)$ is relatively intense but ions are also present at the same m/z value in the mass spectra of hexoses.

3.2.2.8. Differentiation of α- and β-anomers. Identification of anomers by EI-MS is generally difficult to achieve [11]. However, mass spectra of TMSi methyl hexopyranosides show definite and reproducible differences with respect to the relative abundance of the A_2 ion $(M^{\cdot+} - CH_3O^{\cdot} - TMSiOH$, m/z 361) which is about twice as intense for the α-anomer as for the β-anomer. Since these derivatives are expected to favour the same $^4C_1$ chair conformation [26], ease of loss of the C-1 attached methoxyl group appears to be affected by the configuration at the carbon atom. Such differences have been experienced in the EI mass spectra of permethylated glycosides of glucose, mannose and galactose [24].

The above extensive study of the EI mass spectra of TMSi methyl glycosides is summarized in Table 3, where selected ions helpful for differentiation between sugar classes and isomeric forms are reported, thus illustrating the efficiency of the EI-MS technique. We wish to distinguish sugars according to (i) their classes (i.e., pentoses, hexoses, deoxyhexoses, hexuronic acids and related compounds), (ii) their ring size, (iii) their epimeric forms, inside a class, and (iv) their anomeric configuration. The criteria in Table 3 provide unambiguous answer to (i) and (ii) but do not allow one to distinguish between epimeric forms of hexoses (iii), and do not meet (iv) except for hexoses.

In order to remove these uncertainties in classification, a complementary study using NH_3 CI-MS was undertaken.

3.3. Ammonia chemical ionization mass spectrometry

As expected the NH_3 CI mass spectra are simple and easy to interpret. Some representa-

Table 3
Selected ions in the EI mass spectra which allow differentiation between sugar classes and tautomeric forms

m/z	467	451	435	423	391	377	365	363
Compounds	H	H	H	UA	UA	H	P, 4M	DH, UA
m/z	361[a]	349	347	345	333	319[b]	317	290[b]
Compounds	H	P, 4M	DH	H	P, 4M	DH, H, UA, **UL**	UA	P, DH, H
m/z	287	275	273	259	257	247	234	230
Compounds	UL, H	P, 4M	DH, UA	P, 4M, UL	DH	UA	UA, **4M**	P, H, UA 4M, **UL**
m/z	217[b]	204[b,c]	159	146	133[b]			
Compounds	**P, H, DH, UA**, 4M, **UL**	**P, H, DH, UA, 4M**	P, H, DH, **UA, 4M**, UL	P, H, DH, **UA, 4M**, UL	**P, H, DH, UA, 4M**, UL			

Abbreviations: P = pentoses; H = hexoses; DH = deoxyhexoses; UA = uronic acids; UL = glucurono-6,3-lactone; 4M = 4-O-methyl glucuronic acid. When the relative intensity of a peak is higher than 10% of the base peak, the corresponding abbreviation is in bold type.

[a] In addition, m/z 361 ion allows α/β differentiation (see text).

[b] These ions can be used to distinguish furanoses and pyranoses.

[c] The relative intensity of this ion is >30% for pyranoses and <6% for furanoses.

tive examples of each structural type of compounds examined are shown in Fig. 4.

Intense ions observed in the high-mass range enable the molecular mass to be determined unambiguously. These significant peaks correspond to the protonated molecular ion $[MH]^+$, the ammonium adduct ion $[M + NH_4]^+$, together with fragment ions arising from consecutive losses of methanol and trimethylsilanol molecules from the latter two. Possible routes for their formation are illustrated in Fig. 5. Although $[MH]^+$ and $[M + NH_4]^+$ are major positive ions commonly formed under the usual CI source operating conditions, substituted ions are likely to be encountered when ammonia is used as the reagent gas. Upon NH_3 CI of oligosaccharides and glycoconjugates in general, such a process leads to the cleavage of the glycosidic bond with addition of ammonia [27]. It would be reasonable that ions empirically ascribed to $[M + NH_4 - CH_3OH]^+$ in Fig. 5 could arise from the same mechanism, i.e. the substitution of the anomeric methoxyl group by ammonia. Eventually, peaks at *m/z* 204 and 217 were detected, although less intense than in the EI spectra. Their relative intensity allows the assignment of pyranoside and furanoside structures, in a similar way to that proposed from the EI data.

In the following, NH_3 CI mass spectra, summarized in Table 4, will be considered in turn within each set of stereosiomers.

TMSi derivatives of methyl xylosides and methyl arabinosides may be differentiated by comparing the ions at *m/z* 381 and 349, which are more intense in the spectra of methyl xylosides. Differences in the relative abundance of the adduct ion (*m/z* 398) can be used to distinguish between α- and β-forms within a given anomeric pair. Thus, the β-anomers of methyl arabinosides yield a stronger peak at *m/z* 398 than did the α-anomers, the reverse relationship being observed with methyl xylopyranosides.

Results gained from the TMSi methyl glycosides of 6-deoxyaldoses are difficult to rationalize since the chromatographic peaks of methyl β-fucosides co-elute. However, the contribution of methyl β-fucofuranoside within the result chromatographic band should be considered to be negligible, as indicated by the *m/z* 204/217 intensity ratio (compared with the corresponding ratio for methyl α-fucopyranoside). If the occurrence of this minor furanose form is disregarded, the TMSi derivatives of methyl rhamnopyranosides and methyl fucopyranosides can be differentiated by examination of the relative abundance of the adduct ion, which is produced with a greater intensity from the fucose derivatives. Methyl α-fucofuranoside is readily identified owing to its *m/z* 204/217 intensity ratio, but the most noteworthy feature of its NH_3 CI mass spectrum is the relatively weak peak at *m/z* 363 compared with that obtained from the isomeric pyranosides. Differences within a given anomeric pair reside in the $[M + NH_4]^+$ ion of β-anomers showing a more intense peak than that of the α-anomers.

Individual components produced on methanolysis of aldohexoses can be easily identified by their NH_3 CI mass spectra of their TMSi derivatives. Thus, the $[M + NH_4 - CH_3OH]^+$ ion (*m/z* 468) is the base peak in the spectra of methyl mannosides, whereas the methyl pyranosides of glucose and galactose give a base peak at *m/z* 361. The extent of fragmentation of the $[M + NH_4 - CH_3OH]^+$ ion allows one to distinguish the methyl glucosides from the methyl galactosides. Indeed, the consecutive losses of three trimethylsilanol molecules from this ion appears more pronounced in the case of methyl glucopyranosides than with methyl galactosides, leading to intense diagnostic ions at *m/z* 288 and 198. In addition, the relative intensity of the $[MH - CH_3OH]^+$ ion strongly differs according to the starting sugar: its relative abundance ranges between about 76% to 91% for pyranosides and furanosides of galactose, whereas it does not exceed about 37% and 4.5% in the spectra of methyl mannosides and methyl glucosides, respectively. Once again, furanose forms of methyl galactoside can be identified using the *m/z* 204/217 intensity ratio. Another noticeable difference is the greater intensity of the peak observed at *m/z* 271 when compared with that obtained from isomeric methyl hexopyranosides. Independently, anomeric forms of methyl gluco-

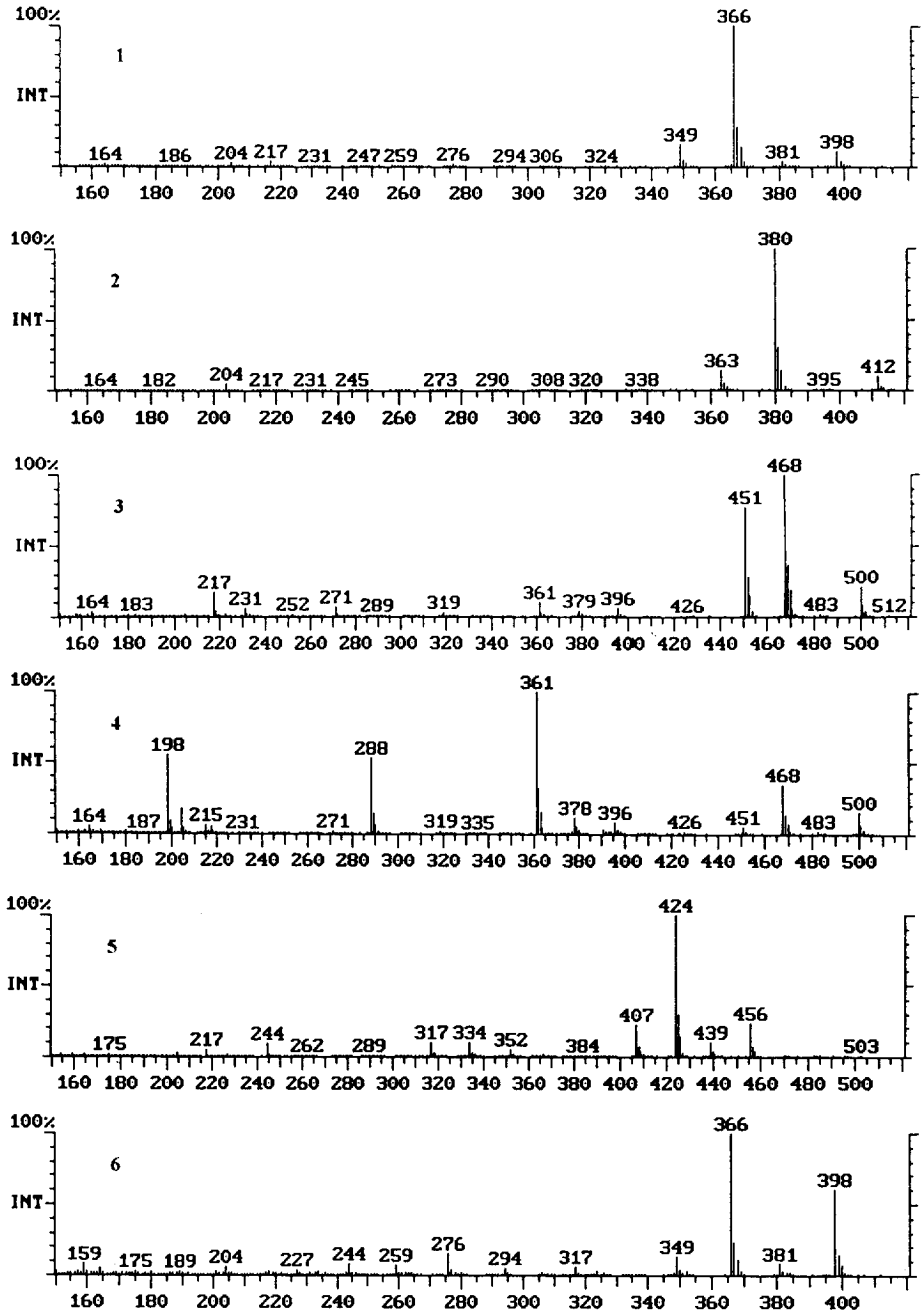

Fig. 4. NH_3 CI mass spectra of TMSi methyl glycosides: (1) methyl β-arabinopyranoside; (2) methyl α-rhamnopyranoside; (3) methyl α-galactofuranoside; (4) methyl α-glucopyranoside; (5) methyl (methyl α-glucopyranosid)uronate; (6) methyl (methyl α,β-4-O-methyl glucopyranosid)uronate (first isomer eluted).

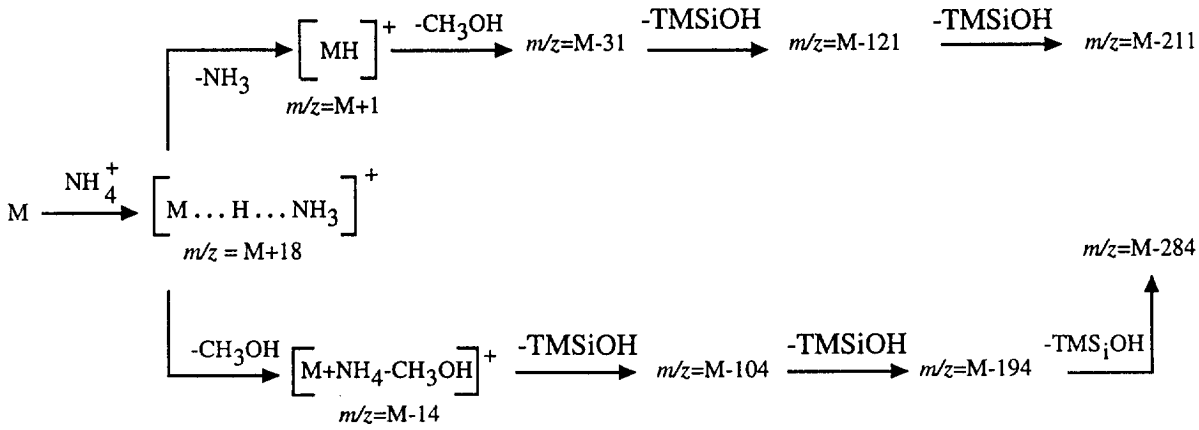

Fig. 5. Main fragmentations of TMSi methyl glycosides in NH_3 CI-MS.

sides and methyl galactosides yield different spectra with a higher relative abundance of the adduct ion for β-anomers. On the other hand, both α- and β-anomers of methyl mannosides give similar spectra. The only differences consist in a slightly higher intensity of the peaks at m/z 500 and 361. However, it is our experience that such minor differences which do not exceed the reproducibility of mass spectral measurement, should not be considered as a reliable criterion for α/β differentiation.

The NH_3 CI mass spectra recorded from TMSi 1-methyl glycoside 6-methyl esters of glucuronic and galacturonic acids were sufficiently different to identify each of them. These compounds are unambiguously differentiated by comparing, for instance, the pattern shown by the peaks at m/z 439, 407 and 244. Differentiation between anomers is also possible, owing to the presence of the m/z 407 and 424 ions, which are always more intense for α- than for β-anomers.

The mass spectra of methyl glucurono-6,3-lactone derivatives are very simple, consisting essentially of a prominent adduct ion (m/z 352). These two compounds give a similar profile in the peak range, except for the relative intensity of the protonated molecular ion (m/z 335), which is slightly higher for the α-anomer.

4-O-methylglucuronic acid and pentose derivatives are structural isomers. Confusion between members of these carbohydrate classes is precluded by significant differences in the NH_3 CI spectra. Hence, the second GC peak of 4-O-methylgucuronic acid yields a spectrum whose base peak is located at m/z 398 ($[M+NH_4]^+$), whereas the substituted ion (m/z 366) is observed as the most intense in the pentose series. The first-eluted isomer of 4-O-methylglucuronic acid is easily identified owing to an intense m/z 276 ion (15% relative abundance). The same ion is measured at less than 6% for the remaining isomeric derivatives. A series of ions including m/z 398, 366 and 276 allow easy differentiation of 4-O-methylglucuronic acid anomers.

As indicated by the foregoing observations, changes in the relative intensities of prominent ions formed upon NH_3 CI reflect differences in the stereochemistry of the studied monosaccharides. With respect to that particular point, NH_3 CI-MS clearly appears more effective than EI-MS for the unequivocal identification of stereoisomeric sugars. Such advantages have been previously reported by Murata and Takahashi [14] when pertrimethylsilylated derivatives of glucose, mannose and galactose were examined. Significant differences were found between the NH_3 CI mass spectra of these compounds. Hence, epimeric sugars were differentiated by the structure of the base peak. It was also found that β-anomers gave an ammonium adduct ion always greater in intensity than did the α-anomers. We observed the same feature in the spectra of methyl glucoside and methyl galactoside derivatives, but no significant

Table 4
Relative intensities and m/z values (>150) of characteristic ions in the NH_3 CI mass spectra of TMSi methyl glycosides.

Methyl glycoside[a]	Structure[b]	M_r	m/z (relative intensity, %)										
			$[M+NH_4]^+$	$[M+NH_4-CH_3OH]^+$	$[M+NH_4-CH_3OH-TMSIOH]^+$	$[M+NH_4-CH_3OH-2\,TMSIOH]^+$	$[M+NH_4-CH_3OH-3\,TMSIOH]^+$	$[MH]^+$	$[MH-CH_3OH]^+$	$[MH-CH_3OH-TMSIOH]^+$	$[MH-CH_3OH-2\,TMSIOH]^+$	204	217
Arabinose 1	α-f	380	398 (9.6)	366 (100.0)	276 (0.5)	186 (0.3)	96	381 (1.2)	349 (5.6)	259 (2.2)	169 (0.2)	0.4	4.9
Arabinose 2	β-p	380	398 (11.3)	366 (100.0)	276 (2.0)	186 (0.6)	96	381 (4.1)	349 (16.5)	259 (0.9)	169 (0.2)	2.5	3.4
Arabinose 3	α-p	380	398 (2.1)	366 (100.0)	276 (1.9)	186 (0.6)	96	381 (6.1)	349 (15.1)	259 (0.9)	169 (0.3)	2.4	3.5
Arabinose 4	β-f	380	398 (59.9)	366 (100.0)	276 (0.8)	186	96	381 (1.7)	349 (8.3)	259 (3.7)	169 (0.3)	1.2	6.0
Xylose 1	α-p	380	398 (27.6)	366 (100.0)	276 (1.5)	186 (1.0)	96	381 (10.4)	349 (26.5)	259 (1.4)	169 (0.5)	5.1	3.4
Xylose 2	β-p	380	398 (13.4)	366 (100.0)	276 (1.7)	186 (1.1)	96	381 (19.1)	349 (31.5)	259 (1.7)	169 (0.4)	5.8	3.7
Rhamnose 1	α-p	394	412 (4.6)	380 (100.0)	290 (0.8)	200 (0.2)	110	395 (0.9)	363 (24.0)	273 (0.5)	183 (0.2)	4.6	0.7
Rhamnose 2	β-p	394	412 (13.3)	380 (100.0)	290 (1.3)	200 (0.2)	110	395 (2.9)	363 (31.9)	273 (0.9)	183 (0.5)	6.0	1.5
Fucose 1	α-f	394	412 (13.2)	380 (100.0)	290 (0.6)	200 (0.1)	110	395 (0.5)	363 (7.0)	273 (1.7)	183 (0.7)	0.3	4.6
Fucose 2	α-p	394	412 (17.3)	380 (100.0)	290 (0.4)	200 (0.4)	110	395 (0.8)	363 (30.6)	273 (1.2)	183 (0.3)	7.0	1.3
Fucose 3	β-f + β-p	394	412 (55.8)	380 (100.0)	290 (0.4)	200 (0.4)	110	395 (1.9)	363 (34.1)	273 (1.1)	183 (0.5)	9.1	2.0
Mannose 1	α-p	482	500 (18.5)	468 (100.0)	378 (6.2)	288 (2.4)	198 (3.2)	483 (0.9)	451 (37.3)	361 (44.5)	271 (0.5)	24.2	5.8
Mannose 2	β-p	482	500 (19.4)	468 (100.0)	378 (6.2)	288 (2.5)	198 (3.1)	483 (1.3)	451 (37.7)	361 (45.8)	271 (0.6)	26.4	5.9
Galactose 1	α-f	482	500 (20.8)	468 (100.0)	378 (1.2)	288 (0.4)	198 (0.8)	483 (0.5)	451 (77.6)	361 (9.3)	271 (6.4)	1.2	16.8
Galactose 2	α-p	482	500 (5.5)	468 (47.1)	378 (4.4)	288 (9.7)	198 (17.2)	483 (9.1)	451 (90.7)	361 (100.0)	271 (0.8)	31.8	8.2
Galactose 3	β-f	482	500 (74.9)	468 (100.0)	378 (2.6)	288 (1.2)	198 (1.7)	483 (0.8)	451 (76.2)	361 (20.0)	271 (16.0)	2.4	29.2
Galactose 4	β-p	482	500 (32.7)	468 (43.4)	378 (4.6)	288 (8.9)	198 (14.3)	483 (8.9)	451 (86.9)	361 (100.0)	271 (0.3)	35.9	8.6
Glucose 1	α-p	482	500 (14.6)	468 (35.0)	378 (10.3)	288 (52.7)	198 (54.7)	483 (1.1)	451 (4.5)	361 (100.0)	271 (1.8)	17.4	5.1
Glucose 2	β-p	482	500 (42.1)	468 (31.6)	378 (9.8)	288 (51.9)	198 (65.6)	483 (2.0)	451 (4.5)	361 (100.0)	271 (1.7)	24.1	6.1
Glucurono-6,3-lactone 1	α-f	334	352 (100.0)	330	240	150 (1.4)		335 (5.2)	303	213	123		0.9
Glucurono-6,3-lactone 2	β-f	334	352 (100.0)	330	240	150 (0.2)		335 (1.0)	303 (0.6)	213 (0.3)	123	0.2	0.4
Glucuronic acid 1	β-p	438	456 (100.0)	424 (29.5)	334 (5.2)	244 (5.2)	154 (0.9)	439 (4.5)	407 (11.2)	317 (6.6)	227 (1.0)	3.4	4.8
Glucuronic acid 2	α-p	438	456 (23.1)	424 (100.0)	334 (10.0)	244 (8.9)	154 (1.1)	439 (9.9)	407 (21.8)	317 (9.5)	227 (1.1)	2.8	4.5
Galacturonic acid 1	α-f	438	456 (100.0)	424 (65.6)	334 (0.5)	244 (0.6)	154 (0.4)	439 (0.8)	407 (43.3)	317 (3.1)	227 (1.1)	1.2	7.9
Galacturonic acid 2	β-f	438	456 (100.0)	424 (14.7)	334 (0.4)	244 (0.8)	154 (0.7)	439 (1.1)	407 (9.5)	317 (1.3)	227 (0.4)	1.1	4.0
Galacturonic acid 3	α-p	438	456 (100.0)	424 (57.5)	334 (2.1)	244 (1.4)	154 (1.3)	439 (85.2)	407 (67.5)	317 (2.8)	227 (0.8)	9.3	7.2
Galacturonic acid 4	β-p	438	456 (100.0)	424 (44.2)	334 (1.8)	244 (1.5)	154 (0.8)	439 (73.6)	407 (49.0)	317 (4.1)	227 (1.2)	12.2	9.1
4-O-Methylglucuronic acid 1	α,β-p	380	398 (60.5)	366 (100)	276 (15.1)	186 (1.9)		381 (7.8)	349 (12.9)	259 (6.5)	169 (1.3)	5.8	1.9
4-O-Methylglucuronic acid 2	α,β-p	380	398 (100)	366 (21.1)	276 (5.3)	186 (1.9)		381 (2.9)	349 (3.8)	259 (3.4)	169 (0.7)	3.7	1.5

differences were found between the spectra of α- and β-mannosides. Westmore and Alauddin [27] reviewed various applications of NH_3 CI-MS for the assignment of stereochemistry and differentiation of isomers taken in several classes of biologically significant compounds. These numerous examples indicate that stereochemical factors, e.g., charge stabilization via intramolecular hydrogen bonding, are likely to affect the extent of the competitive processes involved in the ionization and decomposition of the analyte molecules. Determination of the detailed ion–molecule reactions or unimolecular ion decompositions mechanisms appears necessary to account for the behaviour of a given set of stereoisomers. Such additional work, which remains to be carried out in the case of TMSi derivatives of methyl glycosides, should be of great interest in explaining the present experimental observations.

3.4. Analysis of an ink sample from a seventeenth century parchment

Analysis of ink constituents is usually carried out with the aim of providing valuable information for the preservation or restoration of ancient writings. GC–MS is the method of choice for such investigations, as the amount of sample available is generally limited to a maximum of 1 mg.

Many types of inks have been used in the past, their composition now being well documented [28]. In this field, our work has concentrated on organic constituents of metallo-gallic inks, long known to have been historically widely used. In contrast to other classes of hand-written inks, they are composed of a tannin of plant origin (usually a gallotannin) associated with either a copper or iron sulfate salt. The resulting complexation of tannic acids with copper or iron ions is responsible for the black or brown colour of the ink. The chemical constituents of gallotannins have been already identified by hydrolysis or methanolysis [29,30], yielding tannic acids (namely gallic acid, quinic acid and shikimic acid) and smaller amounts of carbohydrates. The preparation of a metallo-gallic ink involved a third component acting as binding agent. This could be of either proteinic, lipidic or glucidic nature. Plant gums have frequently been used as binding agents. Typically, gum arabic was employed but gum tragacanth or gum from the "prunus" species (e.g., plum tree gum) are likely candidates.

A 0.2-mg ink sample, scraped from a seventeenth century parchment, was used for this study. The chromatogram is shown in Fig. 6. All the identifications reported were made using retention times and EI mass spectra. As a preliminary, a blank experiment was carried out, using the same amount of unused parchment. Only a few compounds were detected in small amounts, but tannic acids or sugars where not found.

Methanolysis of the ink sample yielded monosaccharides as major components, thus proving the presence of a glucidic-type binding agent. A gum may be further assumed since key compounds such as uronic acids (glucuronic acid, 4-O-methylglucuronic acid) were detected. Methyl esters of quinic acid and gallic acid where also found and identified by comparison with GC–EI-MS analysis of derivatized standards. The gallic acid derivative yielded a clear EI mass spectrum, including intense diagnostic ions (m/z 400 $[M]^{\cdot+}$ and 281 $[M^{\cdot+} - CH_3^{\cdot} - Si(Me)_4]^+$) [31], which offers an unambiguous and sensitive method of identification. In the case of the quinic acid derivative, characteristic fragment ions were found at m/z 479 ($[M^{\cdot+} - CH_3^{\cdot}]^+$), 404 ($[M^{\cdot+} - TMSiOH]^+$) and 345 ($[M^{\cdot+} - CO_2CH_3^{\cdot} - TMSiOH]^+$). The presence of tannic acids together with glucose is positive evidence of a gallo-tannin [1] and so indicates clearly the metallo-gallic origin of the ink. In order to determine the botanical source of the gum employed, reference gum samples suspected as potential binding agents were also analysed. Fig. 6 compares the carbohydrate patterns obtained after methanolysis of these materials, which appear readily distinguishable by comparison of their chromatographic profiles. When compared with the investigated ink sample, the most closely related chromatogram is obtained from gum arabic, but significant differences are observed,

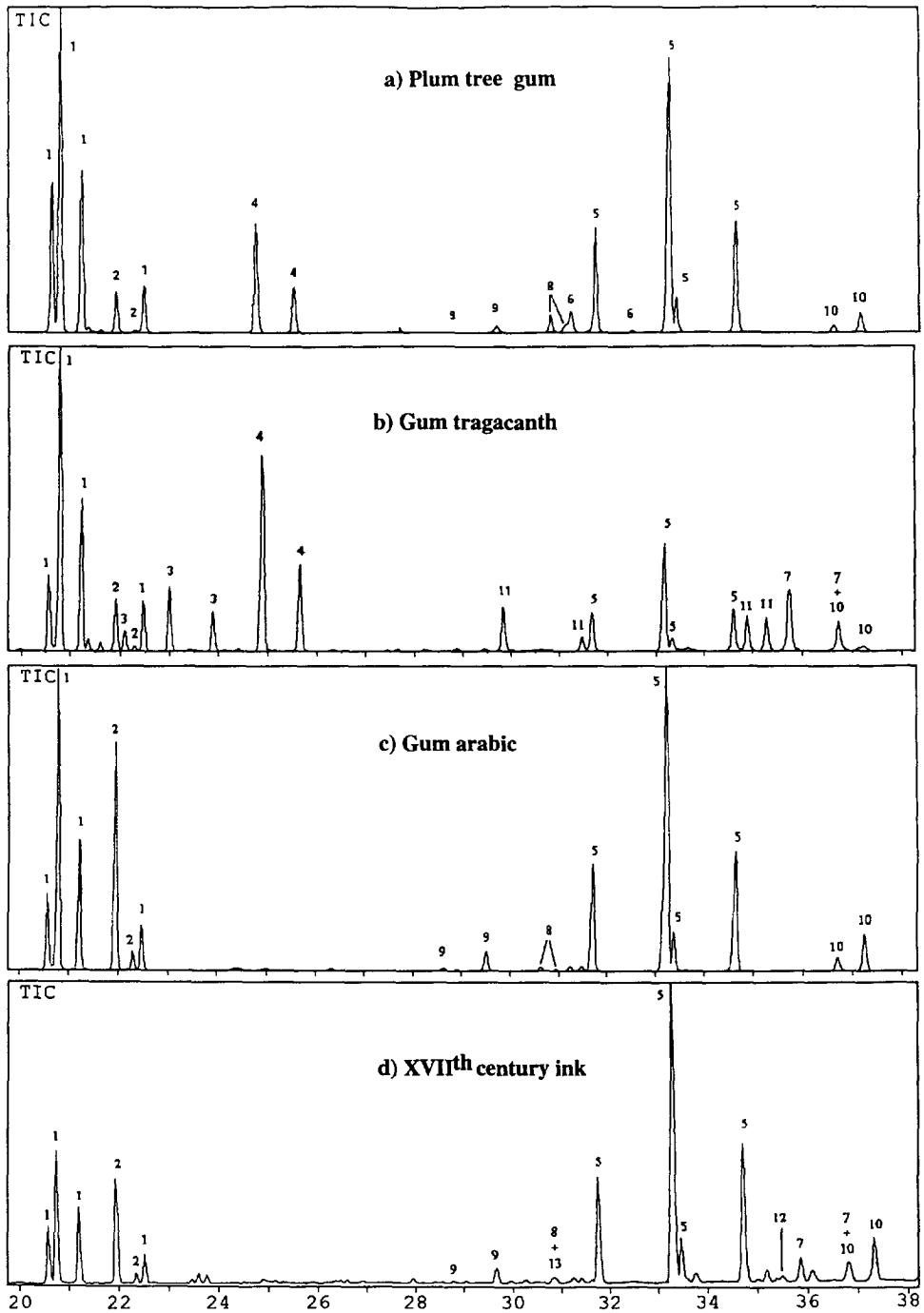

Fig. 6. GC–EI-MS analysis of trimethylsilylated methanolysis products from (a–c) reference gum samples and (d) from a seventeeth century ink sample. Peaks: 1 = arabinose; 2 = rhamnose; 3 = fucose; 4 = xylose; 5 = galactose; 6 = mannose; 7 = glucose; 8 = 4-O-methylglucuronic acid; 9 = glucurono-6,3-lactone; 10 = glucuronic acid; 11 = galacturonic acid; 12 = gallic acid; 13 = quinic acid. Elution time is in minutes.

namely in the presence of glucose in the ink sample and the smaller proportions of arabinose and rhamnose relative to that of galactose. As previously discussed, glucose probably originates from a gallotannin, and should not be considered for comparison purposes.

Some hypotheses can be put forward to account for the differences in the arabinose and rhamnose contents. It is possible that sugars liberated from the gallotannin may confuse the identification of a gum based on monosaccharide ratios. A complementary study provided some evidence to refute this hypothesis. An ink sample prepared according to a Middle Ages recipe (gum arabic being the binding agent) was submitted to the usual analytical procedure. As a result, the obtained chromatographic profile was typical of gum arabic [32].

In a further experiment, the same ink preparation was spotted on to an authentic parchment and left for a few weeks under the ambient conditions of the laboratory. As a significant decrease in the arabinose to galactose ratio was observed, we concluded that glucidic material adsorbed on a parchment may undergo considerable compositional alterations. Chemicals used for the manufacture of the parchment are probably responsible for this degradation process. In contrast, it is our experience that under suitable storage conditions, recent and "ancient" (e.g., collected 100–200 years ago) gum arabic samples are identical by monosaccharide analysis [32]. Thus, the present results leave some uncertainty about the actual botanical source of the binding agent precursor.

Overall, the ink sample investigation can be considered to be satisfactory since crucial information such as the metallo-gallic origin of the sample and the glucidic nature of the binding agent employed have been deduced.

4. Conclusion

The results obtained demonstrate the practical applications of GC–MS for the identification of a wide range of carbohydrate components in plant gums. EI-MS analysis of TMSi methyl glycosides, which give well resolved peaks in capillary columns, allows structural features such as the ring size, nature and position of substituents of the sugar molecule to be determined. CI-MS with ammonia as the reagent gas makes possible the measurement of the molecular mass, thus confirming the EI data. In some cases, CI-MS is very helpful, particularly for the identication of unknown compounds or compounds not available as standards (e.g., 4-O-methylglucuronic acid). Further, the differences between NH_3 CI mass spectra are large enough to distinguish stereoisomeric sugars by pattern comparison.

This study was limited to the neutral and acid monosaccharides contained in plant gums. Complementary work is in progress to check the applicability of the method for the analysis of ketoses and other members of the aldose and uronic acid series.

The successful identification of the constituents used for the preparation of a seventeenth century ink sample was achieved at the submilligram level. Hence the sensitivity of the technique appears suitable to meet the needs of analytical studies on small samples.

The three reference gum samples investigated are readily distinguishable by comparison of the chromatographic profiles. We now intend to examine other gum exudates obtained from as many species as possible in the hope that it may be possible to identify each of them with the same ease. In addition, this methodical approach is currently being applied to the systematic study of a wide variety of natural substances, including gum resins, resins, plant waxes and other materials commonly found in artisic and archeological contexts.

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