Chemical Ionization (Methane) Mass Spectrometry of Sugars and Their Derivatives

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Chemical ionization mass spectrometry (CI-MS) is an effective tool for the identification of sugars and their derivatives, often component parts of natural products. By use of methane as the reagent gas, several classes of sugars including aldoses, fructoses, di- and polysaccharides, deoxy sugars, alcohols, acids, lactones, amines, amides, and glycosides were investigated. The $[MH]^+$ ion was generally present along with a pattern of fragmentation ions that could be used to deduce the structures.

INTRODUCTION

The structural elucidation of biologically active natural products has often involved the identification of component sugars, their derivatives, and various glycosides. Electron impact mass spectrometry (EI-MS) has been used for obtaining structural information on small quantities of carbohydrate derivatives (Budzikiewicz et al., 1964; Dougherty et al., 1974; Horton et al., 1974; Radford and Dejongh, 1980; Harrison, 1983). Peracetates and trimethylsilyl derivatives are particularly useful for this purpose and can provide detectable molecular ions for even tetrasaccharides, provided that the MS mass range is adequate. However, the high energies used for ionization sometimes cause extensive fragmentation so that the small mass fragments dominate. Other disadvantages to the use of EI-MS with carbohydrate derivatives include preparation time, incompleteness of the reaction, and possible rearrangements. As a result, carbohydrates have continued to be the subject of investigations using a number of advanced mass spectral techniques including variations of positive and negative chemical ionization and fast atom bombardment. High-resolution nuclear magnetic resonance spectrometry in various modes has also been successfully applied for structure elucidation.

In the initial stages of work to determine the structure of a carbohydrate, a glycoside, or some related structure, identification of the basic units can give valuable information that may contribute to a later solution. Because, as previously stated, EI-MS of carbohydrates and derivatives frequently gives complex fragmentations with low molecular weight intensities, CI-MS was investigated. The first indication of the potential came from the publication of the CH_4 CI-MS of 2-deoxy-D-ribose (Fales et al., 1969). Subsequently, a variety of carbohydrates have been examined by CI using primarily proton-transfer reagents (Horton et al., 1974; Harrison, 1983) such as methane, isobutane, and ammonia. Ammonia gives strong molecular ions, but it is counterindicated for the inlet and source of some units.

However, because CI-MS data have not been made available in comprehensive published registries as with EI-MS, we first became aware of the potential of CI-MS during our isolation of apigenin 7-*O*-glucoside from crimson clover *Trifolium incarnatum* L. in which we obtained the CH_4 fragmentation pattern m/z 163, 145, 127, and 108 from the sugar residue, identical with that of authentic glucose (Waage and Hedin, 1985).

When biologically active compounds are isolated, glycosides and various other derivatives of carbohydrates are frequently encountered. Thus, a data base with which unknown spectra could be compared is needed. Even though isobutane has been shown to give stronger molecular ions (Harrison, 1983), methane was selected because it was found to provide strong spectra with a variety of aglycons, and it gave definitive fragmentation patterns with the sugars, normally including the molecular ions.

This paper gives tabular CI-MS (CH_4) fragmentation data of 56 sugars and sugar derivatives. Some effects of structure could be correlated with the fragmentation patterns.

MATERIALS AND METHODS

CI Techniques. A Hewlett-Packard 5985-B quadrupole mass spectrometer was used to obtain positive CI spectra with methane as the reagent gas. The experimental conditions were as follows: the source pressure was 3×10^{-4} Torr, the electron energy was 235 eV, and the source temperature was 150 °C. The probe temperature was raised from ambient to 350 °C over 10 min. Spectra were obtained on solids, generally the average of three analyses in which differences were quite minor.

Procurement. All compounds were obtained from one of the following sources: United States Biochemical Corporation, Cleveland, OH; Fluka Chemika-Bio Chemika, Ronkonkoma, NY; ICN Biochemicals, Cleveland, OH; Calbiochem, Los Angeles, CA; K and K Laboratories, Plainview, NY; E. M. Laboratories, Elmsford, NY; and Sigma Chemical Co., St. Louis, MO. The structures can be found in the Fluka catalog or in the *Merck Index* (1983).

RESULTS AND DISCUSSION

Tables I–V list the CI-MS (CH_4) spectra of some aldoses, ketoses, polysaccharides, sugar derivatives, and glycosides. With CI-MS (CH_4) spectra, in general, ions of $[M + 1]^+$, $[M + 16]^+$, $[M + 29]^+$, and $[M + 41]^+$ are often seen, but with these sugars, only the $[M + 1]^+$ ion was generally evident. The $[M + 1]^+$ ion can be the result of self-protonation, which also sometimes gives an $[M - 1]^+$ ion.

The aldoses (Table I) all give a $[M + 1]^+$ ion, though small, except for D-(−)-erythrose. Their major features are the losses of 1, 2, and 3 H_2O. With the aldotetroses and aldopentoses, the $[MH - H_2O]^+$ ion is the base peak. With the aldohexoses, the $[MH - H_2O]^+$ ion is the base peak for all except D-(+)- and D-(−)-glucose, where the $[MH - 2\ H_2O]^+$ ion is the base peak.

An evaluation of the stereochemistry of the aldohexoses revealed that the 2, 3, and 4 carbons of D-(+)- and

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Table I. CI-MS (CH_4) Fragmentations (m/z) of Aldoses[a]

	MW	fragmentations	features
		Aldotrioses	
DL-glyceraldehyde	90	91 (100), 73 (5), 61 (29)	[MH − 1 H_2O]$^+$
		Aldotetroses	
D-(−)-erythrose	120	103 (100), 85 (4), 75 (3), 61 (6)	[MH − 1,2 H_2O]$^+$
L-(+)-threose	120	121 (4), 103 (100), 85 (17), 73 (5), 61 (18)	
		Aldopentoses	
D-(−)-ribose	150	151 (3), 133 (100), 115 (13), 97 (2), 73 (18)	[MH − 1,2,3 H_2O]$^+$
D-(−)-lyxose	150	151 (3), 133 (100), 115 (15), 97 (3), 73 (14)	
L-(+)-arabinose	150	151 (2), 133 (100), 115 (15), 97 (2), 73 (26)	
D-(+)-xylose	150	151 (2), 133 (100), 115 (40), 97 (7), 73 (30)	
		Aldohexoses	
D-(+)-glucose	180	181 (1), 163 (76), 145 (100), 127 (38), 115 (7)	[MH − 1,2,3 H_2O]$^+$
D-(−)-glucose	180	181 (2), 163 (82), 145 (100), 127 (61), 115 (15)	
L-(−)-glucose	180	181 (1), 163 (100), 145 (96), 127 (43), 115 (8)	
D-(+)-galactose	180	181 (3), 163 (100), 145 (80), 127 (25), 115 (9)	
D-(+)-mannose	180	181 (3), 163 (100), 145 (76), 127 (33), 115 (8)	
L-(−)-idose	180	181 (16), 163 (100), 145 (64), 127 (17), 115 (3)	
D-(+)-altrose	180	181 (1), 163 (100), 145 (85), 127 (32), 115 (11)	
D-(+)-talose	180	181 (31), 163 (100), 145 (49), 127 (14), 115 (4)	

[a] The $[MH]^+$ ion is underlined.

Table II. CI-MS (CH_4) Fragmentations (m/z) of Ketoses

	MW	fragmentations	features
		Ketopentoses	
L-(−)xylulose	150	247 (85), 229 (92), 133 (100), 115 (82), 97 (48)	[MH − 1,2,3 H_2O]$^+$, [MMH − 1,2,3 H_2O]$^+$
D-(−)ribulose	150	265 (100), 247 (26), 133 (43), 115 (5), 87 (2)	
		Ketohexose	
D-(−)-fructose	180	325 (10), 289 (7), 163 (100), 145 (78), 127 (33)	[MH − 1,2,3 H_2O]$^+$, [MH − 1,2,3,4 H_2O]$^+$
		Ketoheptoses	
D-(−)-sedo-heptulosan	210	385 (4), 193 (49), 175 (73), 157 (100), 139 (7)	[MH − 1,2,3,4 H_2O]$^+$, [MMH − 1,2,3,4 H_2O]$^+$
D-(−)-gluco-heptulose	210	385 (3), 193 (54), 175 (72), 157 (100), 139 (23)	

D-(−)-glucose possess OH groups that are equatorial, whereas with the other sugars there are various combinations of equatorial and axial OH groups at these positions. The e,e,e configuration is evidently more conducive to "multiple" dehydration. This feature therefore has diagnostic value for distinguishing between glucose and other hexoses. However, CI-MS analysis of a diglycoside such as a galactosidyl glycoside, for example, would not give definitive data, because the pattern would reflect an average of both sugars, so that supplemental chromatographic and NMR data would need to be acquired.

On the other hand, xylosyl glucosides are occasionally encountered as diglycosides, and evidence for their presence could be deduced from ion fragments at m/z 133, 115, and 97 from xylose and at m/z 163, 145, and 127 from glucose.

Among the four aldopentoses, only D-(+)-xylose has equatorial hydroxyl groups at C-2, C-3, and C-4. While xylose also has the same base peak m/z, 133 $[MH - H_2O]^+$, as the other three aldopentoses (in contrast to D-(+)- and D-(−)-glucose with the other aldohexoses), it has a considerably stronger m/z, 115 $[MH - 2H_2O]^+$, than the other aldopentoses, 40% vs 15%. Also, the intensity at m/z 97 is stronger than that of the other three aldopentoses. These differences may be sufficiently large to be considered in assignment of their structures.

Only five ketoses could be procured for evaluation: two ketopentoses, one ketohexose, and two ketoheptoses (Table II). None showed $[MH]^+$ ions; however, they dimerized, and losses from both the monomers and dimers were observed $[MH - 1, 2, 3, \text{and } 4\ H_2O]^+$ $[MMH - 1, 2, 3, \text{and } 4\ H_2O]^+$. With fructose, $[MH - 1\ H_2O]^+$ is the base peak as with all of the aldohexoses except D-(+)- and D-(−)-glucose. While the fragmentation pattern of fructose is very similar to that of sucrose (Table III), it differs markedly in that the $[MH - 1 \text{ and } 2\ H_2O]^+$ fragments from sucrose are much stronger.

Table III. CI-MS (CH_4) Fragmentations (m/z) of Di- and Polysaccharides

	MW	fragmentations	features
		Disaccharides	
D-(+)maltose (glu 1→4 glu)	342	325 (2), 307 (2), 163 (41), 145 (100), 127 (71)	[MH − 1,2,3,4 H_2O]$^+$, [M/2H − 1,2,3 H_2O]$^+$
D-(+)trehalose (glu 1→1 glu)	342	325 (1), 289 (1), 163 (45), 145 (100), 127 (57)	
gentiobiose (glu 1→6 glu)	342	163 (16), 145 (100), 127 (18)	
D-(+)meliobiose (gal 1→6 glu)	342	289 (3), 163 (83), 145 (100), 127 (38)	
D-(+)lactose (gal 1→4 glu)	342	325 (1), 307), 163 (33), 145 (100), 127 (40)	
palatinose (glu 1→6 glu)	342	289 (2), 163 (16), 145 (65), 12 7 (100)	
sucrose (glu 1→2 glu)	342	325 (44), 289 (43), 163 (100), 145 (57), 127 (48)	
		Polysaccharides	
D-(+)raffinose (gal 1→6 glu →2 fru)	504	325 (1), 289 (2), 163 (89), 145 (95), 127 (100)	m/z 163, 145, 127
D-(+)stachyose (gal 1→6 gal 1→6 glu 1→2 fru)	666	325 (1), 289 (5), 163 (60), 145 (64), 127 (100)	

Table III. CI-MS (CH_4) Fragmentations (m/z) of Di- and Polysaccharides

	MW	fragmentations	features
		Disaccharides	
D-(+)maltose (glu 1→4 glu)	342	325 (2), 307 (2), 163 (41), 145 (100), 127 (71)	[MH − 1,2,3,4 H_2O]$^+$, [M/2H − 1,2,3 H_2O]$^+$
D-(+)trehalose (glu 1→1 glu)	342	325 (1), 289 (1), 163 (45), 145 (100), 127 (57)	
gentiobiose (glu 1→6 glu)	342	163 (16), 145 (100), 127 (18)	
D-(+)meliobiose (gal 1→6 glu)	342	289 (3), 163 (83), 145 (100), 127 (38)	
D-(+)lactose (gal 1→4 glu)	342	325 (1), 307), 163 (33), 145 (100), 127 (40)	
palatinose (glu 1→6 glu)	342	289 (2), 163 (16), 145 (65), 12 7 (100)	
sucrose (glu 1→2 glu)	342	325 (44), 289 (43), 163 (100), 145 (57), 127 (48)	
		Polysaccharides	
D-(+)raffinose (gal 1→6 glu →2 fru)	504	325 (1), 289 (2), 163 (89), 145 (95), 127 (100)	m/z 163, 145, 127
D-(+)stachyose (gal 1→6 gal 1→6 glu 1→2 fru)	666	325 (1), 289 (5), 163 (60), 145 (64), 127 (100)	

CI-MS spectra were obtained on seven disaccharides, a trisaccharide, and a tetrasaccharide (Table III). No $[MH]^+$ ions were noted. The fragmentation patterns were, with the exception of that for sucrose, not discernibly different

Table IV. CI-MS (CH_4) Fragmentations (m/z) of Sugar Derivatives[a]

	MW	fragmentations	features
		Deoxy Sugars	
			$[MH - 1,2,3\ H_2O]^+$, $[MMH - 1,2\ H_2O]^+$
2-deoxy-D-ribose	134	117 (100), 99 (36), 81 (2)	
2-deoxy-D-glucose	164	147 (97), 129 (100), 111 (54)	
L-(+)-rhamnose	164	293 (1), 275 (1), 147 (100), 129 (61), 111 (4)	
L-(−)fucose	164	293 (10), 275 (3), 147 (100), 129 (49), 111 (5)	
		Alcohols	
			$[MH]^+$, $[MH - 1,2,3,4\ H_2O]^+$, $[MMH]^+$, $[M/2H - 1,2,3,4\ H_2O]^+$
i-erythritol	122	245 (71), 124 (4), 123 (100), 105 (21), 87 (38)	
D-(+)-arabitol	152	305 (35), 153 (100), 135 (17), 117 (41), 99 (31)	
m-ribitol	152	305 (34), 153 (100),135 (19), 117(46), 99 (34)	
m-galactitol(dulcitol)	182	365 (10), 183 (100),165 (16), 147(24), 129 (59)	
D-(+)-mannitol	182	365 (3), 183 (100), 165 (27), 147 (34), 129 (61)	
i-inositol	180	361 (19), 181 (76), 163 (59), 145 (19), 127 (67), 109 (100)	
maltitol (4-*O*-α-gluco-pyranosyl-D-sorbitol)	344	183 (88), 165 (35), 147 (49), 129 (100), 111 (26)	
		Acids	
			$[MH]^+$, $[MH - 1,2\ H_2O]^+$, $[MH - CO]^+$, $[M - 1]^+$ $[MMH]^+$, $[MMH - 2,4\ H_2O]^+$
glyoxylic acid	74	93 (10), 75 (100), 61 (2)	
L-(+)-tartaric acid	150	301 (1), 151 (60), 133 (10), 123 (7), 105 (100)	
D-(−)-tartaric acid	150	301 (2), 151 (56), 133 (11), 123 (11), 105 (100)	
D-(+)-glucuronic acid	194	317 (7), 193 (10), 177 (17), 159 (95), 131 (26), 115 (100)	
m-galactaric acid (mucic acid)	210	385 (1), 193 (18), 175 (16), 165 (25), 147 (100), 119 (50)	
		Lactones	
			$[MH]^+$, $[MH - 1,2\ H_2O]^+$, $[MMH - 1,2,3\ H_2O]^+$
D-(+) ribonic acid 1,4-lactone	148	297 (16), 149 (100), 113 (21), 103 (29), 85 (18)	
L-(−) mannono-1,4-lactone	178	357 (2), 179 (59), 161 (4), 143 (8), 133 (100), 115 (11)	
D-(+)-glucurono-6,3-lactone	176	335 (1), 177 (10), 159 (100), 141 (3), 131	
		Amines/Amides	
			$[M]^+$, $[MH]^+$, $[MH - 31, 36]^+$,$[MH - 1,2,3\ H_2O]^+$
D-(+)-glucosamine hydrochloride	215	215 (6), 185 (43), 180 (35), 162 (100), 103 (85)	
D-(+)-galctosamine hydrochloride	215	215 (3), 185 (100), 162 (9), 103 (62)	
N-acetyl-β-D-glucosamine	221	222 (26), 204 (100), 186 (86), 168 (32), 126 (34)	
N-acetyl-β-D-mannosamine	221	222 (10), 204 (71), 186 (100), 168 (37), 126 (57)	

[a] The $[MH]^+$ ion is underlined.

Table V. CI-MS (CH_4) Fragmentations (m/z) of Selected Glucosides[a]

glycoside	MW	fragmentations	features
esculetin 6-glucoside	340	341 (1), 179 (100), 163 (1), 145 (3), 127 (3)	$[MH]^+$
quercitrin (quercetin 3-rhamnoside)	448	303 (23), 287 (6), 147 (1), 129 (36), 111 (5)	
apigenin 7-glucoside	432	329 (11), 301 (56), 271 (93), 163 (11), 145 (54), 127 (100)	$[MH + 29, 41]^+$
hesperidin (hesperitin 7-rutinoside)	610	304 (15), 303 (100), 287 (14), 147 (1), 129 (1)	

[a] Underlined 341 indicates $[MH]^+$ ion; underlined 179 indicates $[MH]^+$ ion of the aglycon.

from those of the ketoses (Table III). Fragments arising from $[MH - 1, 2, \text{and } 3\ H_2O]^+$ and $[M/2\ H - 1, 2, \text{and } 3\ H_2O]^+$ were observed. The base peak was m/z 145, $[M/2\ H - 2\ H_2O]^+$ in all instances where there was a glucose with a free C-1. This was pronounced where both units were glucose. However, with palatinose and sucrose, where the glucose C-1 was glycosidated with fructose, the base peak was different: m/z 127 $[M/2\ H - 3\ H_2O]^+$ for palatinose and m/z 163 $[M/2\ H - H_2O]^+$ for sucrose. Evidently the glu 1→6 fru linkage is less stable than glu 1→2 fru.

Neither raffinose nor stachyose was sufficiently stable to provide molecular ions; in fact, m/z 127, arising from the loss of 3 H_2O from the monomer, was the base peak. Thus, it is evident that only limited information can be obtained from CI-MS (CH_4) analysis of polysaccharides.

Table IV lists CI-MS spectra of several classes of sugar derivatives including four deoxy sugars, seven alcohols, five acids, three lactones, and four amines or amides. Two of the deoxy sugars showed some evidence of dimerization. The fragmentation patterns were otherwise similar to those of the aldoses and ketoses, giving fragments of m/z $[MH - 1, 2, \text{and } 3\ H_2O]^+$ and the dimers $[MMH - 1$ and $2\ H_2O]^+$.

With all of the alcohols except maltitol, a strong $[MH]^+$ ion was present, often the base peak. Fragments resulting from dimerization $[MMH]^+$ were also prominent. Other fragments present were m/z $[MH - 1, 2, 3, \text{and } 4\ H_2O]^+$ and, from maltitol, $[M/2\ H - 1, 2, 3, \text{and } 4\ H_2O]^+$.

The five selected acids exhibited more differences than similarities, so that recognition of the acids as a class appears impossible. Fragments representing m/z $[MH]^+$, $[MH - 1, 2\ H_2O]^+$, $[M - 1]^+$, $[MH - CO]^+$, $[MMH]^+$, and $[MMH - 2$ and $4\ H_2O]^+$ were observed. D-(−)- and L-(+)-tartaric acid gave nearly identical spectra, both of which could be confused with the spectra of aldopentoses (m/z 151, 133, 115), except that the acids dimerized, giving $[MMH]^+$. D-(+)-Glucuronic acid was the only sugar that gave a $[M - 1]^+$ ion. The lactones also gave diverse spectra with dimerization again evident. Fragmentations included $[MH]^+$, $[MH - 1, 2\ H_2O]^+$, $[MMH]^+$, and $[M - CO_2]^+$.

D-(+)-Glucosamine hydrochloride and D-(+)-galac-

tosamine hydrochloride gave small but distinct molecular ions for the amine salts. The $[M - 30]^+$ fragment (m/z 185) is the base peak of D-(+)-galactosamine hydrochloride and a prominent fragment of D-(+)-glucosamine hydrochloride. Both also showed successive losses of water. *N*-Acetyl-β-D-glucosamine and *N*-acetyl-β-D-mannosamine give prominent $[MH]^+$ ions and a succession of fragments attributable to the successive losses of 1, 2 and 3 H_2O. The strong ion at m/z 126 could arise from ring opening adjacent to the oxygen and subsequent cleavage between carbons 4 and 5.

Table V gives CI-MS (CH_4) fragmentation patterns of four glycosides. Methane is obviously deficient in not providing molecular ions; nevertheless, considerable information was obtained by using this mode. All of the glycosides provided the $[MH]^+$ ion of the constituent aglycon. With the monoglycosides, the identity of the sugar was evident from the fragmentation pattern. With hesperitin 7-rutinoside, only the fragmentation pattern from rhamnose, the "end" sugar, was evident. In identification work, these data could be quickly clarified because initial chromatographic evidence would have indicated the presence of a diglycoside. Subsequent hydrolysis and analysis of the sugar residue would reveal the presence of both rhamnose and glucose from which it could be inferred that glucose was directly linked to the aglycon.

In summary, CI-MS is an effective tool for identification work on sugars as such or as components of natural products. The advantages of this method are that it is easy, generally accessible, and rapid, and it should give an insight about the structure of the carbohydrate.

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