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CHEMICAL-IONIZATION MASS SPECTROMETRY OF METHYLATED HEXITOL ACETATES*

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ABSTRACT

Many of the stereoisomers of methylated hexitol acetates having the same arrangement of *O*-methyl and *O*-acetyl groups yield markedly different chemical-ionization (c.i.) mass spectra. For example, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylgalactitol yield clearly distinguishable c.i. mass spectra. The observed differences are reproducible. Hence, chemical-ionization mass spectrometry, when combined with analytical gas chromatography and electron-impact (e.i.) mass spectrometry, is of value in identifying the methylated alditol acetates obtained during methylation analysis of polysaccharides and other complex carbohydrates. The c.i. mass-spectral data that differentiate many of the possible partially methylated glucitol, galactitol, and mannitol acetates are presented. In addition, examples are presented that demonstrate the ability of c.i. mass spectrometry to characterize previously unidentified methylated hexitol acetates.

INTRODUCTION

A convenient and powerful method of determining glycosyl linkage-compositions of polysaccharides is based on the conversion of a methylated polysaccharide into its corresponding, alditol acetates[1]. These volatile derivatives may frequently be separated by analytical gas chromatography[1]. Electron-impact (e.i.) mass spectrometers, coupled directly to the effluent of gas chromatographs, have provided an essential tool for identification of the partially methylated alditol acetates[2]. E.i. mass spectrometry readily distinguishes between all alditols having different arrangements of *O*-methyl and *O*-acetyl groups. However, e.i. mass spectrometry does not differentiate different isomers having the same arrangement of *O*-methyl and *O*-acetyl groups[2]. For example, e.i. mass spectra of the methylated hexitol acetates derived from 3-linked and from 4-linked glucose‡ are quite different, but the e.i., mass spectra

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‡A simplified notation for the methylated hexitol acetates has been adopted. All of the methylated hexitol acetates studied in this paper are derived from aldohexopyranose residues and have *O*-acetyl

of the derivatives of 4-linked glucose, 4-linked galactose, and 4-linked mannose are indistinguishable. These latter types of isomers can, at present, be distinguished only if they can be separated by gas chromatography and their retention times compared to standards. A number of these isomers have not been separated, and an even larger number of isomers cannot be separated on a single gas chromatography column.

The c.i. mass spectra of most of the possible methylated hexitol acetates that can be derived from the three aldohexopyranoses, glucose, galactose, and mannose, are presented in this paper. All of these hexitol acetates are monodeuterated at C-1. For many given arrangements of *O*-methyl and *O*-acetyl groups, the derivatives of the three aldohexopyranoses may be distinguished on the basis of their c.i. mass spectra. Therefore, c.i. mass spectrometry is a useful method for identifying some of the methylated alditol acetates that cannot be identified by e.i. mass spectrometry.

RESULTS

The major quantitative differences, if differences exist, in the c.i. mass spectra of all of the hexitol isomers occur in the relative intensities of the M+1, M+1−32, and M+1−60 ions. All of the methylated hexitol acetates yield these ions. The *m/e* value of each ion depends on the molecular weight of the methylated hexitol acetate being examined. Portions of the c.i. mass spectra of the methylated hexitol acetates derived from 4-linked glucose and of 4-linked galactose, using isobutane as the ionizing gas, are presented in Fig. 1. The ions of these derivatives at *m/e* 390, 352, 320, and 292 correspond to M+39, M+1, M+1−32, and M+1−60, respectively. No other ions are present in an abundance of greater than 5% of the most abundant ion. The data of Table I compare the relative intensities of the M+1, M+1−32, and M+1−60 ions for many of the methylated hexitol acetates that can be derived from mannose, galactose, and glucose. The relative intensities of these ions were found to be quite sensitive to source temperature. The M+1−60 and M+1−32 ions predominate at higher source-temperatures, whereas the M+1 ions are enhanced by lower source-temperatures. A source temperature of 145° (±5°) was selected for these studies because all three (of these diagnostic) ions are present for most compounds at this temperature.

We have identified two unknown, methylated hexitol acetates with the assistance of c.i. mass spectrometry. The unknowns were obtained during methylation analysis

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groups attached to both O-1 (protected from *O*-methylation by its participation in a glycosidic linkage) and O-5 (protected from *O*-methylation by its participation in the pyranose ring). In the notation adopted, all hydroxyl positions designated as "linked" have *O*-acetyl groups (protected from methylation by glycosidic linkage with another sugar), whereas all of the remaining oxygen atoms (except O-1 and O-5) have *O*-methyl groups. Any aldohexose designated as "terminal" has no "linked" hydroxyl groups. The following examples illustrate the notation: 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylmannitol is referred to as terminal mannose; 1,3-5-tri-*O*-acetyl-2,4,6-tri-*O*-methylglucitol as 3-linked glucose: and 1,2,3,5-tri-*O*-acetyl-4,6-di-*O*-methylgalactitol as 2,3-linked galactose. This notation is useful for the purposes of the present paper, but would require modification for general use encompassing furanoid forms, ketose residues, and other types of component residues.

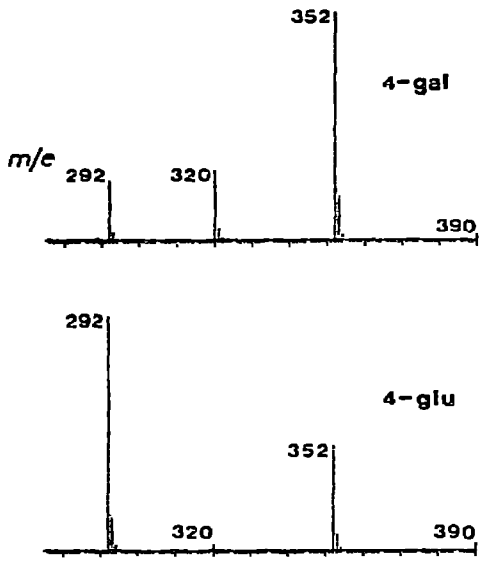

Fig. 1. Portions of the chemical-ionization mass spectra of the methylated hexitol acetate derivatives of 4-linked galactose and 4-linked glucose.

TABLE I

RELATIVE INTENSITIES OF THE M+1, M+1−32, AND M+1−60 IONS OBTAINED FROM VARIOUSLY METHYLATED MONO-DEUTERIUM-LABELED HEXITOL ACETATES[a]

Alditol acetate derivatives		*M+1*[b]	*M+1−32*	*M+1−60*	*Number of spectra averaged*[c]	*Number of sample sources*[d]
	m/e value	*324*	*292*	*264*		
T-Linked[e]	Gal	100	17 (5)	87(18)	5	2
	Glc	9.0(1.8)	1.3(0.4)	100	6	4
	Man	2.6(0.9)	0.9(0.2)	100	7	2
	m/e value	*352*	*320*	*292*		
2-Linked	Gal	100	21 (2)	53 (8)	3	1
	Glc	100	8 (2.4)	39 (5)	4	1
	Man	100	10 (1.4)	32 (5)	6	2
	m/e value	*352*	*320*	*292*		
3-Linked	Gal	98 (5)[f]	7.2(1)	91 (7)	4	2
	Glc	39 (7)	1.8(0.4)	100	6	3
	Man	26 (7)	2.2(0.5)	100	6	2
	m/e value	*352*	*320*	*292*		
4-Linked	Gal	100	31 (9)	28(11)	5	3
	Glc	44(16)	3.2(0.4)	100	5	2
	Man	14 (2)	1.5(0.2)	100	4	2

(*Table continued on p. 242*)

TABLE I (*continued*)

Alditol acetate derivatives		*M+1*[b]	*M+1−32*	*M+1−60*	*Number of spectra averaged*[c]	*Number of sample sources*[d]
	m/e value	*352*	*320*	*292*		
6-Linked	Gal	60 (5.2)	2.1(0.5)	100	4	3
	Glc	71 (7)	2.3(0.4)	100	3	1
	Man	77(16)	3.4(1)	99 (3)[f]	7	3
	m/e value	*380*	*348*	*320*		
2,4-Linked	Gal	100	36 (2)	43 (1)	3	2
	Man	49 (5)	6.5(0.9)	100	3	2
	m/e value	*380*	*348*	*320*		
2,6-Linked	Glc	44 (6)	1.8(0.5)	100	4	1
	Man	47(11)	2.3(0.6)	100	4	2
	m/e value	*380*	*348*	*320*		
3,4-linked	Gal	62 (5)	10.7(1)	100	4	2
	Glc	44 (6)	4.1(1)	100	6	2
	Man	17 (3)	4.1(0.6)	100	4	1
	m/e value	*380*	*348*	*320*		
3,6-Linked	Gal	80 (4)	3.5(0.5)	100	3	2
	Glc	67(14)	2.2(0.4)	100	3	1
	Man	59 (9)	3.9(1.4)	100	4	1
	m/e value	*380*	*348*	*320*		
4,6-Linked	Gal	27 (3)	2.3(0.7)	100	4	3
	Glc	100	6.0(2)	90 (6)	4	2
	Man	100	3.1(0.7)	89(10)	5	2
	m/e value	*408*	*376*	*348*		
3,4,6-Linked	Gal	27 (4)	1.0(1)	100	4	2
	Glc	64 (5)	2.3(0.9)	100	5	2
	Man	22 (4)	2.5(2)	100	4	1

[a]All derivatives had one deuterium atom attached to C-1. [b]The relative intensities presented are the average of several spectra obtained by separate injections on the gas chromatograph. The numbers in parentheses are the standard deviations. [c]This is the number of spectra averaged for the data presented. Each spectrum is from a separate gas-chromatograph injection. [d]The number of separate sources from which each derivative was prepared. Hence, two sources means that that particular derivative was prepared from two completely different sources of the derivative, and their spectra were averaged. [e]T = Terminal. [f]In these derivatives, the base peak was the M + 1 ion in some spectra and the M + 1 − 60 ion in other spectra. Hence, the averaged relative-intensities show no ion peak having a relative intensity of 100.

of oat coleoptile cell-walls. Unknown no. 1 was determined by e.i. mass spectrometry to be a derivative of a 3,4-linked aldohexopyranose, and was identified by its retention time as either the glucose or galactose derivative; the corresponding mannose derivative has a different gas-chromatographic retention-time. Unknown no. 2 was shown by e.i. mass spectrometry to be the derivative of a 3,4,6-linked hexose.

The c.i. mass-spectral data of these unknowns were obtained by using selected ion-monitoring rather than by scanning the entire mass-range from 200–500 atomic-mass units. The mass spectrometer was set to monitor the ions at *m/e* 408, 348, 380, and 320. (The ions at *m/e* 408 and 348 are the M+1 and M+1−60 ions for the

derivatives of the 3,4,6-linked hexoses; the ions at *m/e* 348, 380, and 320 are the M + 1 − 32, M + 1, and M + 1 − 60 ions for the derivatives of the 3,4-linked hexoses.) As the mass spectrometer monitored only four ions, the intensities of these ions were accurately measured and are plotted, *vs* elution time from the gas chromatograph, in Fig. 2. The ion-peaks corresponding to the derivatives of the unknown 3,4- and 3,4,6-linked hexoses are shaded; the other ion-peaks result from other methylated

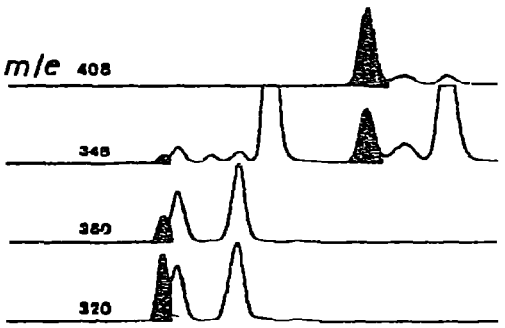

Fig. 2. Mass chromatograms of the methylated alditol acetates resulting from an analysis of oat coleoptile cell-walls. The intensities of four selected ions are plotted as a function of elution time from the gas chromatograph. The mass spectrometer was operated as described in the text. Ions obtained from the derivatives of the unknown 3,4- and 3,4,6-linked aldohexopyranoses are shaded. The intensities of the ion at *m/e* 348 are expanded to 300%.

alditol acetates derived from the coleoptile cell-walls. The area under each ion peak is a measure of the intensity of that ion. The areas were calculated and normalized to the area of the M + 1 − 60 ion peak for each unknown by using a computer program supplied with the g.c.–m.s. system by Hewlett-Packard. Similar analyses were made of authentic derivatives of 3,4-linked galactose, 3,4,6-linked galactose, 3,4-linked glucose, and 3,4,6-linked glucose. The relative intensities of the ions having *m/e* 408, 348, 380, and 320, calculated in this manner, are presented in Table II. The data demonstrate that the 3,4-linked hexose derivative (unknown no. 1) is from glucose and not from galactose. The 3,4,6-linked aldohexose derivative is clearly not from galactose, and reference to Table I shows that it is more likely to be from glucose than from mannose. Comparison of the retention time of this unknown with that of standard compounds confirms that it is, in fact, the derivative of 3,4,6-linked glucose.

A further use of c.i. mass spectrometry is demonstrated by the identification of the methylated hexitol acetates derived from terminal hexoses. Terminal mannosyl and terminal glucosyl residues yield derivatives possessing similar retention-times on all gas chromatography columns reported in the literature. The glucosyl derivative is eluted slightly ahead of the mannosyl derivative on an ethylene glycol adipate, ethylene glycol succinate, and XF-1150 column[3], but these derivatives are not sufficiently separated to afford two peaks. Hence, it is difficult to determine whether a sample contains one or both of the terminal aldohexopyranose derivatives. Even

TABLE II

RELATIVE INTENSITIES OF SELECTED IONS FOR THE METHYLATED HEXITOL ACETATE DERIVATIVES OF SOME STANDARD AND UNKNOWN 3,4-LINKED AND 3,4,6-LINKED ALDOHEXOPYRANOSES[a]

Hexitol derivatives	m/e *408*	m/e *348*	m/e *380*	m/e *320*
3,4-linked Gal	[b]	11.0	66	100
3,4-linked Glc		3.7	33	100
3,4-linked unknown no. 1		4.0	30	100
3,4,6-linked Gal	26.8	100		
3,4,6-linked Glc	53.1	100		
3,4,6-linked unknown no. 2	55.3	100		

[a]These derivatives had one deuterium atom attached to C-1. [b]A blank indicated the ion is not present in the c.i. mass spectra of these hexitol derivatives.

TABLE III

RELATIVE INTENSITIES OF THE M+1, M+1−32, AND M+1−60 IONS[a] (*m/e* 324, 292, AND 264, RESPECTIVELY) IN THE LEADING, MIDDLE, AND TRAILING PORTIONS OF A GAS-CHROMATOGRAPH PEAK KNOWN TO CONTAIN THE DERIVATIVES OF TERMINAL-MANNOSE AND TERMINAL-GLUCOSE

Hexitol derivative	m/e *324*	m/e *292*	m/e *264*
T-Glc[b]	9.0	1.3	100
Leading 25%	7.6	1.0	100
Middle 25%	4.8	1.1	100
Trailing 25%	3.5	0.9	100
T-Man[b]	2.6	0.9	100

[a]These ions had one deuterium atom attached to C-1. [b]Values from Table I. T = Terminal.

TABLE IV

RELATIVE INTENSITIES OF M+1, M+1−32, AND M+1−60 IONS[a] FOR SOME METHYLATED HEXITOL ACETATES OBTAINED BOTH BEFORE AND AFTER CLEANING OF THE ION SOURCE OF THE MASS SPECTROMETER

Hexitol derivative	*Before source cleaning*[b]			*After source cleaning*		
	M+1	*M+1−32*	*M+1−60*	*M+1*	*M+1−32*	*M+1−60*
T-Gal	100	17 (5)	87(18)	100	18	83
T-Glc	9.0 (1.8)	1.3(0.4)	100	7.9	1.3	100
T-Man	2.6 (0.9)	0.9(0.2)	100	2.3	1.0	100
2-Man	100	10 (1.4)	32 (5)	100	10	29
4-Gal	100	31 (9)	28(11)	100	31	26
4-Glc	44(16)	3.2(0.4)	100	48	3.1	100
4-Man	14 (2)	1.5(0.2)	100	15.7	1.1	100
6-Glc	71 (7)	2.3(0.4)	100	64	2.3	100
2,6-Man	47(11)	2.3(0.6)	100	67	2.0	100

[a]These ions had one deuterium atom attached to C-1. [b]The data are from Table I. Numbers in parentheses are standard deviations.

if only one of the terminal aldohexopyranose derivatives is present, its identification cannot be made with certainty from retention-time data alone.

A sample of methylated alditol acetates containing a mixture of the methylated hexitol acetates derived from terminal glucose and terminal mannose was injected into the gas chromatograph and analyzed by isobutane c.i. mass spectrometry. The mass spectrometer was set to monitor selectively the ions at *m/e* values 324, 292, and 264. These are the M+1, M+1−32, and M+1−60 ions of terminal aldohexopyranose derivatives. The three ion-intensities *vs* elution time from the gas chromatograph were plotted and the leading, middle, and trailing 25% portions of each ion-peak were integrated. The areas obtained for the *m/e* 324 and 292 ions were normalized to the value obtained for the area of the *m/e* 264 ion. The results of this analysis (Table III) show that the leading edge of the gas-chromatograph peak is enriched in the derivative of terminal glucose, whereas the trailing edge is enriched in the derivative of terminal mannose.

An effort was made to determine whether the observed differences in c.i. spectra were dependent on the condition of the mass-spectrometer source. After all of the foregoing data had been collected, the source was disassembled, cleaned, and reassembled. Spectra of some of the methylated hexitol acetates were then obtained again. These data from the cleaned source are compared in Table IV with the corresponding data obtained earlier. Essentially all of the values obtained upon a single injection following cleaning of the source are within the standard deviation of the corresponding value obtained by multiple injections of multiple samples prior to cleaning of the source.

DISCUSSION

Our earliest c.i. experiments were made with methane as the ionizing gas. Methane gave large M+1−60 and M+1−32 ions as well as other lower weight, fragment-ions. Differences in the spectra from some aldohexopyranose isomers are apparent with methane ionization. However, use of isobutane as the ionizing gas gives relatively large M+1 ions and differentiates between the derivatives of the aldohexopyranose isomers to a greater extent. Hence, isobutane was used for all of the experiments reported here.

It is important that the data of Table I be accurate and reproducible if the observed quantitative differences between the spectra of the stereoisomers of the methylated hexitol acetates are to be of value. The following steps have been taken to demonstrate that the data are, in fact, useful:

(*1*) The data for each isomer in Table I were obtained on many different days. The intensity of each ion is the average from at least three spectra taken at different times.

(*2*) Most of the isomers were prepared from two or three different starting-materials. The identity and purity of each starting material was checked by gas chromatography and by c.i. mass spectrometry of the gas-chromatograph peaks.

Furthermore, some of the compounds (the derivatives of the three terminal and three 4-linked aldohexopyranoses) were introduced into the mass spectrometer on two different g.c. columns (see Methods). These precautions make it highly unlikely that any of the differences reported in Table I resulted from the presence of contaminants.

(*3*) The data in Table IV show that the spectra remained unchanged, even after the mass spectrometer had been totally disassembled, cleaned, and reassembled.

Even though the data in Table I are reproducible, we did find that the relative intensities of the ions are sensitive to the ion-source temperature. At 130°, the *m/e* 324 ion of the derivative of terminal mannose becomes about 6% rather than 2.4% of the base *m/e* 264 ion. Therefore, the c.i. spectra of the derivative of terminal mannose at 130° begins to resemble more the c.i. spectrum of the derivative of terminal glucose at 145°. (However, the relative intensity of the *m/e* 324 ion of the derivative of terminal glucose is also increased at 130° as compared to 145°.) Because the c.i. spectra are sensitive to source temperature, it is best to compare the spectrum of an unknown derivative with the spectra of known derivatives that have been injected into the g.c.–m.s. just prior to, or following, injection of the unknown. This procedure was followed for the examples presented in Tables II and III.

The c.i. spectra are relatively independent of the isobutane pressure. Nevertheless, care was taken to use the same pressure of isobutane (see Methods) in all experiments in order to minimize any undetected differences.

The data presented in this paper demonstrate that the three terminal aldohexopyranose derivatives may each be identified from their isobutane c.i. mass spectra. This is especially useful for identification of the terminal mannosyl and glucosyl derivatives, as none of a large variety of g.c. columns that we have tested separate these derivatives effectively. Furthermore, the derivatives of all three 4-linked and all three 3,4-linked hexoses may be distinguished solely on the basis of their c.i. spectra. The 2-linked, 3-linked, and 4,6-linked galactosyl derivatives may be distinguished clearly from the corresponding glucosyl and mannosyl derivatives, although the glucosyl and mannosyl derivatives cannot be distinguished from each other. Finally, the 2,4-linked galactosyl and 2,4-linked mannosyl derivatives may also be distinguished from one another by their c.i. mass spectra.

An added benefit of c.i. mass spectrometry of the methylated alditol acetates is that the molecular weight of the M + 1 ion unambiguously delineates the number of methyl and acetyl groups present in that molecule. In addition, the c.i. mass spectra of the derivatives of 2,3,4-linked aldohexopyranoses yield the *m/e* values, 408, 376, 348 (data not presented), expected for the M + 1, M + 1 − 32 and M + 1 − 60 ions, respectively; these *m/e* values identify these compounds as penta-*O*-acetyl-mono-*O*-methylhexitols. This is useful, as the e.i. spectra from the 2,3,4-linked aldohexopyranose are somewhat difficult to interpret, as the only *O*-methyl group of these isomers is at the end of the alditol chain.

It would be interesting to understand why stereoisomers of methylated alditol acetates, which have the same sequence of methyl and acetyl groups along the hexitol

chain, yield different c.i. mass spectra. The observed differences in the fragmentations are rather surprising, as these isomers are open-chain compounds and free to rotate along the carbon backbone. We offer no explanation for the demonstrated differences.

Chemical-ionization studies of both underivatized and variously derivatized carbohydrates have been reported[4–6]. Depending on the ionizing gas, most of the spectra consist of M + H and M + NH_4 ions and/or simple elimination-products from these ions. No differences in the spectra of different monosaccharide isomers have been reported. Reinhold *et al.*[7] have presented the e.i. mass spectra of per-*O*-trimethylsilyl carbohydrate boronate derivatives. These spectra do allow determination of the relative stereochemistry of the hydroxyl groups. However, this technique can only be carried out with unmethylated sugars and, therefore, is not useful for glycosyl linkage-analyses of polysaccharides.

METHODS

Partially methylated, alditol acetates were prepared from oligo- or polysaccharides of known structure by sequential methylation, hydrolysis, reduction with sodium borodeuteride, and acetylation as described earlier[8]. These carbohydrates consisted of nigeran and sophorose (Koch–Light Laboratories); laminaran, gentiobiose, and guar gum (Sigma Chemical Company); sycamore cell-walls[8]; and (1→4)-β-D-galactan isolated from citrus pectin (Sunkist)[9]. Other methylated alditol acetates were synthesized by formation of the alditol acetates of intentionally undermethylated methyl α-D-mannopyranoside (Sigma), methyl β-D-galactopyranoside (Koch–Light), and methyl β-D-glucopyranoside (Koch–Light). All of the methylated alditol acetates were identified by g.c. retention-times[8] and e.i. mass spectrometry[2]. All of the mass spectra presented were obtained by mass-spectrometric analysis of the effluent of a gas chromatograph, and all of the mass spectra have a single deuterium atom at C-1 of the hexitols, which was introduced during reduction of the corresponding hexoses with sodium borodeuteride.

All mass-spectral analyses were performed with a Hewlett-Packard gas chromatograph–mass spectrometer system (Model 5980A) coupled to a Hewlett–Packard data system (Model 5933A). The samples were introduced through the gas chromatograph, which was fitted with columns containing ethylene glycol succinate, ethylene glycol adipate, and XF-1150 on Gas Chrom P[3] or columns containing 3% OV-275 and 0.4% XF-1150 on Gas Chrom Q[10]. The gas-chromatograph oven was temperature-programmed for all analyses. The program was varied according to the derivatives being separated. In all instances, the maximum temperature was 190°.

Helium, at a flowrate of 30 ml/min, was used as the carrier gas for e.i. mass spectrometry. Methane, at a flowrate of 10 ml/min, served as both the carrier gas and the ionizing gas for methane c.i. mass spectrometry. Helium, at a flowrate of 10 ml/min, was the carrier gas for isobutane c.i. mass spectrometry.

Isobutane, the ionizing gas, was introduced directly into the source of the mass

spectrometer as described by a Hewlett–Packard application note[11]. The pressure of the isobutane in the source was approximately 0.5 torr and the pressure of the helium approximately 1.0 torr. Under these conditions, essentially all of the charged ions originate from the isobutane[11]. The pressure of the isobutane, although approximately 0.5 torr, was always adjusted so that the ratio of the isobutane *m/e* 39 and 43 ions was approximately 2:1.

The ion-source temperature of the mass spectrometer was maintained at 145° (±5°), the electron energy at 48 V, and the filament at 15 mA. The mass spectrometer was usually set to scan from 200–500 atomic-mass units. However, the spectra in Fig. 1 were scanned from 60 to 500 atomic-mass units and the data presented in Tables II and III were obtained by selectively focusing on the atomic masses indicated in the text. The scan speed was usually 1.8 sec/scan.

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