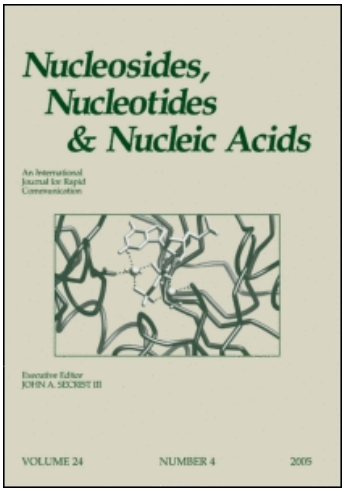

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Fast ATOM Bombardment Mass Spectra of Nucleosides. Comparison with Electron Impact and Chemical Ionization Mass Spectra

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FAST ATOM BOMBARDMENT MASS SPECTRA OF NUCLEOSIDES. COMPARISON WITH ELECTRON IMPACT AND CHEMICAL IONIZATION MASS SPECTRA

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Abstract. The fast atom bombardment (FAB) mass spectra of the eight major nucleosides found in RNA and DNA, pseudouridine and 2',3'-O-isopropylidene adenosine are described and compared to EI, CI, and desorption chemical ionization (DCI) spectra reported in the literature or obtained in this laboratory. Both positive and negative ion FAB spectra are reported. The FAB spectra are simple and provide unambiguous molecular weight information along with structurally significant fragment ions. Mechanisms of ion formation are thought to closely parallel those suggested earlier to be operating in the CI mode. Advantages and disadvantages of FAB relative to the standard ionization modes are discussed.

INTRODUCTION

The recent introduction of fast atom bombardment (FAB)[1] as an ionization technique in mass spectrometry has proven to be of fundamental significance in the analysis of thermally labile and/or nonvolatile molecules.[2,3] Although field desorption (FD)[4] and ^{252}Cf-plasma desorption (PD)[5] have been used with great success to obtain mass spectra of samples intractable to the more classical electron-impact (EI) and chemical ionization (CI) modes, these methods are experimentally complex and require expensive modifications to existing mass spectrometers. In contrast, sample preparation[6] and instrumental modifications are relatively simple with FAB and results have been shown to be readily reproducible between laboratories.[7] These and other advantages[8] make FAB a method of general utility with particular application to the mass spectral analysis of highly polar, thermally labile molecules of biochemical and biomedical interest.

The production of normally intense protonated molecular ions coupled with the formation of structurally significant fragment ions is an additional important feature of FAB mass spectra. The combination of molecular weight and structural information in the same

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spectrum has been used to sequence oligopeptides[9-11] and for the characterization of oligosaccharides,[12,13] steroid sulfates[14] and a number of other compound classes.[2,3]

Application of FAB to the analysis of nucleic acid components has also received some attention, but not to the degree evidenced in other areas, e.g., peptides. The structure determination of isomeric 2'-deoxydinucleoside monophosphate salts using positive and negative ion FAB in conjunction with MIKES has been described.[15] A brief report has also appeared describing the positive/negative ion FAB spectra of some di-, tri-and modified nucleotide phosphates.[16] The identification of three alkylated nucleotide adducts formed in the reaction of guanosine-5'-monophosphate with the antitumor agent phosphoramide mustard utilized FAB ionization.[17] Other biologically important molecules containing a nucleotide portion in their structures have also been analyzed using FAB.[15,16,18,19] Molecular weight and sequence data on high molecular weight oligodeoxynucleotides are available using negative ion FAB[20] and a method has been described for determining the number of active hydrogens present in nucleotides (and other compound classes) using deuterium labeled glycerol as the matrix.[21] More recently, the FAB spectra of 10 mononucleotides and 11 dinucleoside monophosphates have been described.[22]

In contrast to the literature describing the utility of FAB in the analysis of nucleotides and oligonucleotides is the paucity of data concerning the FAB spectra of nucleosides. Only one brief mention of the positive/negative ion spectra of guanosine and deoxyguanosine has appeared in the literature[22] and a recent paper mentions the use of low and high resolution FAB in the structure confirmation of synthetic pyridine nucleoside analogs.[23] Finally, during preparation of this manuscript a paper appeared describing the spectra of nucleosides obtained using FAB combined with MS/MS analysis.[24] A number of the conclusions arrived at independently in the present work are in agreement with this latter report.

The volatility limits exhibited by many naturally occurring and synthetic nucleoside analogs has long been recognized as a major limitation in the mass spectral analysis of this class of compounds[25,26] and electron impact ionization may or may not provide a usable spectrum. In successful cases, EI is seen to provide a wealth

of structurally diagnostic ions and some indication of molecular weight, if not displaying the molecular ion itself, and the mechanisms of fragmentation in the EI mode have been, for the most part, well established.[25,26] Chemical ionization of the more volatile free nucleosides has also been investigated and found to provide information (molecular weight) complementary to that provided by EI.[27] However, the mass spectral analysis of the less volatile or thermally labile members of this compound class requires either derivatization to form a more volatile analog[28] or ionization by FD[29,30], PD[31] or desorption chemical ionization (DCI)[32-34] in order to obtain usable, structurally relevant mass spectra.

The ability to obtain mass spectral data on large biomolecules containing nucleotide components and to sequence DNA and RNA fragments is of fundamental importance. However, a number of situations require the analysis of the more basic structural units. Examples include the characterization of synthetic nucleoside analogs, the identification and quantitation of nucleosides possessing chemotherapeutic activity and the structure elucidation of nucleosides obtained from natural sources and isolated either as a native nucleoside or derived from hydrolysis of larger units. In order to determine the utility of FAB in providing structurally informative mass spectra of nucleosides and to offer a basis for comparison with spectra obtained using EI and/or CI, the positive and negative ion low resolution FAB spectra of the eight major nucleosides found in RNA and DNA, and some closely related analogs, have been examined. In this preliminary account no attempt has been made to optimize experimental conditions and fragmentation mechanisms or ion structures are suggested based on known pathways observed and established using EI and CI.

Experimental

Nucleosides **1-10**, the glycerol and the sulfolane used for the sample matrices are commercial products purchased from Vega Biochemicals, Tucson, Arizona or from Sigma Chemical Company, St. Louis, Missouri.

Mass spectra were obtained using a Varian MAT 311A mass spectrometer fitted with an Ion Tech 11NF saddle field gun. Xenon was used as the primary beam gas and the atom gun was operated at 8 kV

potential with 1 mA emission current. Samples (1-5 ug) were dissolved in glycerol (5-10 ul) on the stainless steel probe tip, and spectra were acquired at ambient temperature with a scan rate of 25 sec/decade. This slower scan rate is recommended to preserve a good signal/noise ratio and to prevent ion statistics from eliminating low abundance peaks.[35]

The acquired spectra were processed by subtraction of the peaks due to the presence of the glycerol matrix using a Varian SS200 data system.

Results and Discussion

Positive Ion

The positive ion FAB mass spectra of the major ribonucleosides found in RNA, i.e., adenosine (1), guanosine (2), uridine (3) and cytidine (4), and the major 2'-deoxyribonucleosides of DNA, i.e., 2'-deoxyadenosine (5), 2'-deoxyguanosine (6), 2'-deoxycytidine (7) and thymidine (8), were examined. Pseudouridine (9) and 2',3'-O-isopropylidene adenosine (10) are also included to illustrate the behavior of a C-nucleoside and a volatile nucleoside derivative. Adenosine (1) is used as a model compound for the purine nucleosides since good mass spectra of the underivatized sample are obtained in FAB, CI and EI allowing comparison of these three ionization modes, as shown in Figure 1. Likewise, thymidine (8) is the model compound for pyrimidine deoxynucleosides and the mass spectra of 8 are displayed in Figure 2.

The major feature of the positive ion FAB spectra of both the ribosides and deoxyribosides is the simplicity of the spectra and the predominance of the MH^+ and $(B+2H)^+$ ions.[36] Molecular weight assignment is unambiguous in all cases, with identification of the MH^+ ion being confirmed by the appearance of one or more glycerol adduct ions, $(MH+G)^+$, separated by 92d beginning from the MH^+ ion. Unlike CI, peaks 2d above the expected value of MH^+ and $(B+2H)^+$ ions in pyrimidine nucleosides[27] are absent in FAB. The relative intensities of the MH^+ ion in FAB as compared to EI and CI methods are shown in Table 1. The increased intensity of the MH^+ ion observed using FAB relative to the other "soft" ionization methods of CI and DCI may be

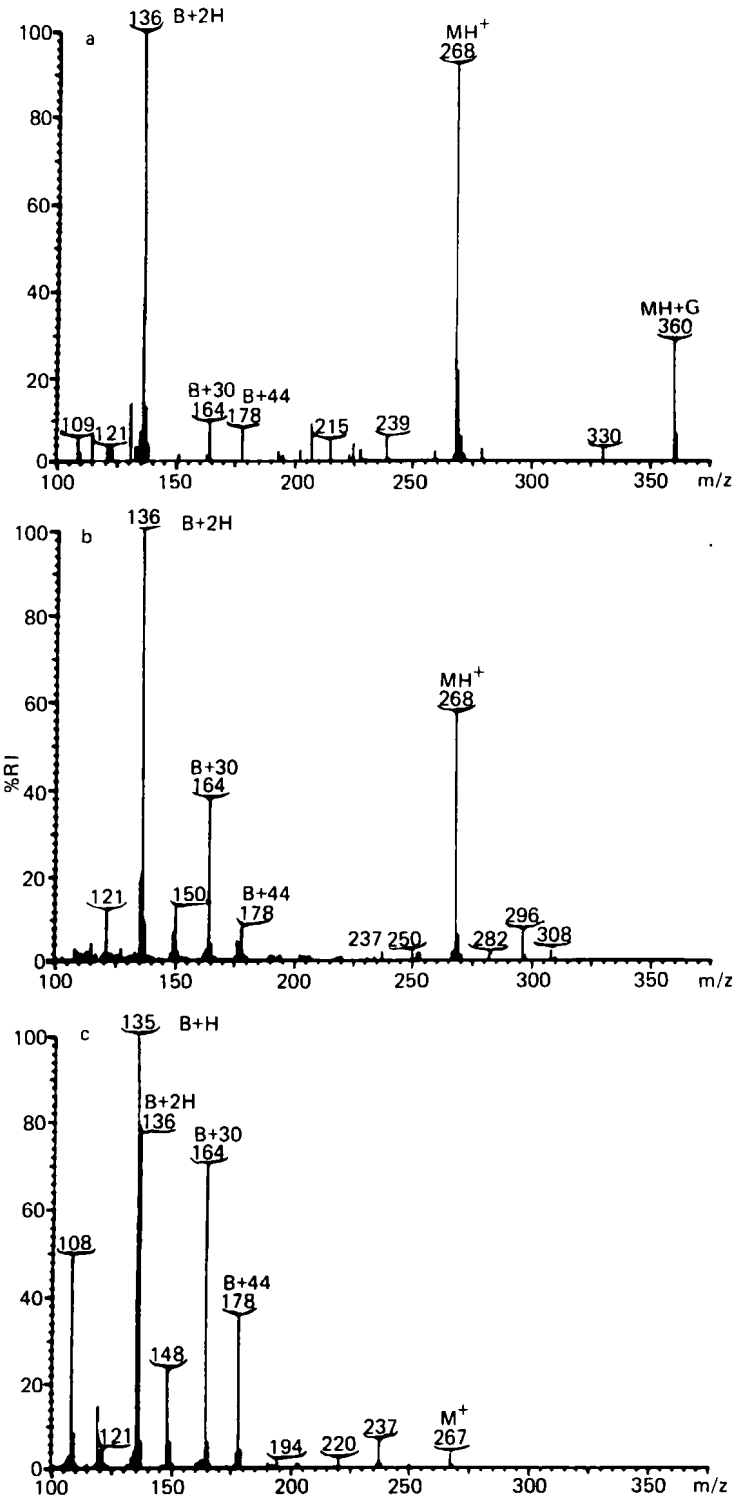


Fig. 1. **Mass spectra of adenosine (1) in a) FAB, b) CI and c) EI ionization modes.**

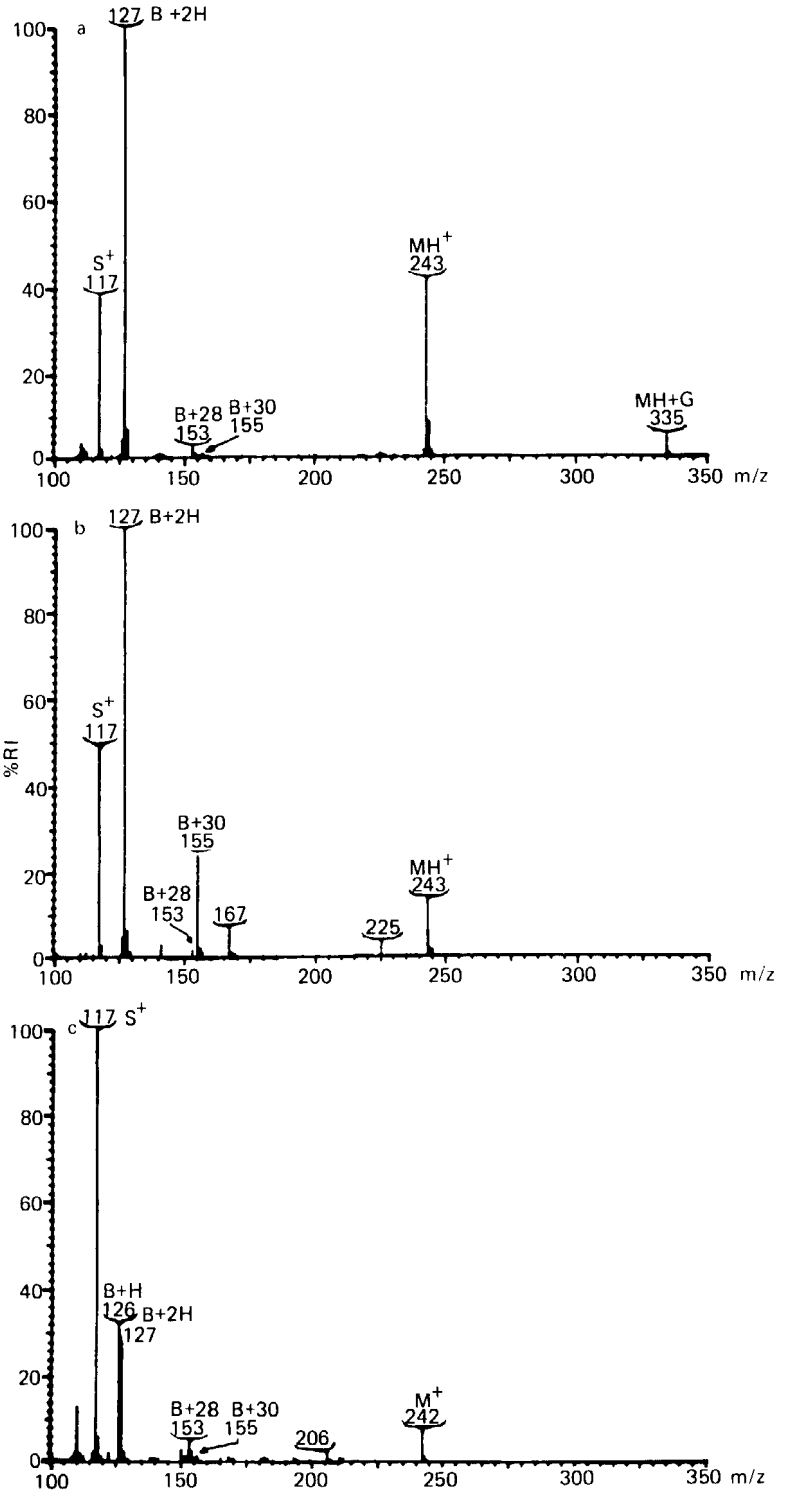


Fig. 2. Mass spectra of thymidine (8) in a) FAB, b) CI and c) EI ionization modes.

ascribed to the method of ionization, the absence of thermal decomposition, or both.

The ability of FAB to provide molecular weight information on samples not amenable to standard EI and CI techniques is most clearly evidenced in the spectra of compounds **2** and **6**. Under ordinary circumstances, both of these nucleosides undergo extensive pyrolysis during heating of the direct insertion probe, which, in the case of **2**, renders the spectrum essentially useless for molecular weight assignment.[37] Desorption CI has provided MH^+ ions of **2** ranging from 17 to 53% relative intensity[32-34] with other strong ions permitting identification of the aglycone m/z 152, $(B+2H)^+$ and sugar (m/z 133, S^+) components of the nucleoside. Attempts to obtain the mass spectrum of **6** using EI, CI and field ionization (FI) have proven unsuccessful. A mass spectrum of **6** has been obtained, however, using a specially prepared probe in the DCI mode.[32] The MH^+ ion observed using these conditions, although readily identifiable, was very weak (0.62% RI) and was accompanied by presumably thermally derived ions at MH^+-18 and MH^+-36. The DCI spectrum is dominated by low mass ions related to the sugar ion (S^+, m/z 117, 83%) by loss of one (m/z 99, 50%) and two (m/z 81, 100%) molecules of water. The use of DCI has the significant disadvantages that the MH^+ ions are transient, experimental conditions are not yet standardized, and the technique has not been widely used for the analysis of nucleic acid components.

In contrast, the positive ion FAB mass spectra of **2** and **6** provide intense MH^+ ions allowing unambiguous molecular weight assignment. A recent paper has also demonstrated intense MH^+ (70% RI for **2** and 50% RI for **6**) and $(B+2H)^+$ ions (100% RI for both) for **2** and **6**,[22] comparable in relative intensity to the results indicated in this study, thus again illustrating the interlaboratory reproducibility of FAB results. In addition, the ions generated using FAB are very long lived and are thus amenable to other mass spectral procedures, including high resolution mass measurements[38] and metastable ion analyses.[35]

Cytidine (**4**) and a number of cytidine analogs are another class of nucleosides which provide weak or nonexistent molecular ions under normal EI conditions.[39] Proper choice of reagent gas in the CI mode[27] or the use of DCI[33] have been shown to produce MH^+ ions for **4** ranging

Table 1: Comparative intensities of the molecular or MH^+ ion and significant fragment ions using FAB, CI and EI techniques in the positive ion mode.[a]

		MH^+[b,c] m/z (%RI)	B+44[d] m/z (%RI)	B+30[e] m/z (%RI)	B+2H m/z (%RI)	S^+ m/z (%RI)	ASSIGNMENT: OTHER m/z (%RI)
Adenosine (1)	FAB	268(92)	178(3)	164(7)	136(100)	133(3)	$(MH+G)^+$:360(28)
	CI	" (59)	" (3)	" (3)	" (100)	" (2)	
	EI	267(20)	" (35)	" (70)	" (78)	" (2)	BH^+:135 (100)
Guanosine (2)	FAB	284(62)	194(5)	180(3)	152(100)	133(2)	$(MH+G)^+$: 386 (12)
	CI	-	-	-	-	-	-
	EI	-	-	-	-	-	-
	DCI	284(30)	(C_4H_{10})[32]	-	152(100)	133(90)	$(S-H_2O)^+$:115(70)
	"	" (17)	(NH_3)[33]	-	" (47)	-	$(S-H)^+$:132(7)
	"	" (54)[34]		-	" (100)	133(30)	$(S-H_2O)^+$:115(30)
Uridine (3)	FAB	245(58)	155(6)	141(<1)	113(100)	133(11)	$(MH+G)^+$: 337 (13)
	CI	" (19)	" (3)	" (17)	" (100)	" (33)	
	EI	244(9)	" (6)	" (20)	" (100)	" (57)	BH^+: 112 (22)
Cytidine (4)	FAB	244(63)	154(4)	140(4)	112(100)	-	$(MH+G)^+$: 336 (12)
	CI	" (2)	" (2)	" (2)	" (100)	133(2)	
	EI	243(1)	" (6)	" (43)	" (100)	" (2)	(B+41): 151 (26)
							BH^+: 111 (46)
Deoxyadenosine (5)	FAB	252(54)	162(7)	164(4)	136(100)	117(4)	$(MH+G)^+$: 344 (8)
	CI	" (31)	" (6)	" (23)	" (100)	" (5)	
	EI	251(10)	" (37)	(12)	" (30)	" (5)	BH^+: 135 (100)
Deoxyguanosine (6)	FAB	268(43)	178(4)	180(1)	152(100)	117(2)	$(MH+G)^+$: 360 (3)
	CI	-	-	-	-	-	
	EI	-	-	-	-	-	
	DCI	268(<1)	(C_4H_{10})[32]	-	152(20)	117(83)	$(S-H_2O)^+$:99(50)
Deoxycytidine (7)	FAB	228(46)	138(6)	140(1)	112(100)	117(5)	$(MH+G)^+$: 320 (5)
	CI	-	" (2)	" (4)	" (100)	" (20)	
	EI	227(2)	" (15)	" (<1)	" (63)	" (15)	BH^+: 111 (100)
							153 (18)
Thymidine (8)	FAB	243(42)	153(2)	155(<1)	127(100)	117(38)	$(MH+G)^+$: 335 (4)
	CI	" (19)	" (2)	" (24)	" (100)	" (49)	
	EI	242(8)	" (4)	" (<1)	" (27)	" (100)	BH^+: 126 (32)
Pseudouridine (9)	FAB	245(100)	155(29)	141(8)	113(15)	-	$(MH+G)^+$: 337 (17)
							$(MH-2H_2O)^+$: 209 (7)
							$(MH-3H_2O)^+$: 191 (2)
							ion a:179 (8)
	CI	" (64)	" (100)	" (19)	" (28)	133(2)	$(MH-H_2O)^+$: 227 (5)
							$(MH-2H_2O)^+$: 209 (54)
							$(MH-3H_2O)^+$:191 (4)
							ion a:179 (20)
	EI	244(<1)	" (8)	" (100)	" (10)	-	$(M-H_2O)^+$: 226 (4)
							$(M-2H_2O)^+$: 208 (6)
							ion a:179 (10)
2',3'-O-isopropylidene adenosine (10)	FAB	308(53)	178(1)	164(5)	136(100)	173(1)	$(MH+G)^+$: 400(4)
							ion b: 292 (<1)
							ion c: 250 (<1)
							ion d: 218 (2)
	FAB[f]	" (64)	" (4)	" (14)	" (100)	" (6)	$(MH+SF)^+$:428(1)
							ion b: 292(4)
							ion c: 250(1)
							ion d: 218(7)
	CI	-	-	-	-	-	
	EI	307(2)	178(2)	164(44)	136(37)	173(3)	ion b: 292(5)
							ion c: 249(5)
							ion d: 218(49)

Table 1 Notes:

a) EI and CI intensity values from reference 47.

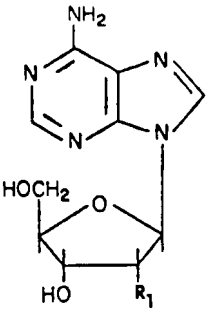
b) Molecular ion species in EI is $M^{+\cdot}$.

c) Methane used as reagent gas in CI mode unless otherwise noted.

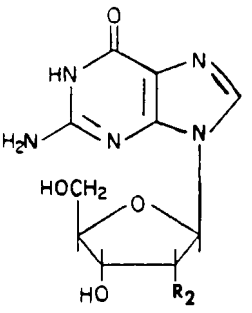
d) In the deoxy series, the ion corresponding to the B+44 ion is the B+28 ion.

e) The relative contributions of $BH{\cdot}C_2H_5$ and $B{\cdot}CH_2O$ to the B+30 ion in the CI mode (methane) are not currently known.

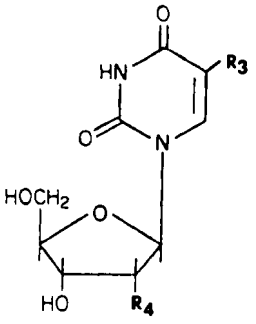
f) Matrix is sulfolane (SF).



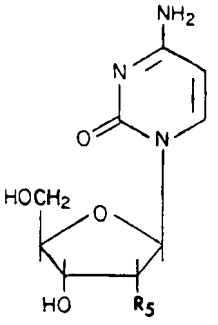
1, R_1 = OH
5, R_1 = H



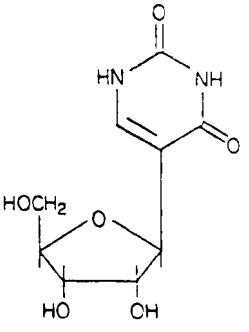
2, R_2 = OH
6, R_2 = H



3, R_3 = H, R_4 = OH
8, R_3 = CH_3, R_4 = H



4, R_5 = OH
7, R_5 = H



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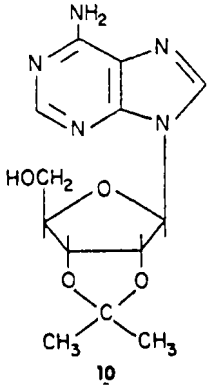


10

in relative intensity from 2% (CI, CH_4) to 100% (DCI, NH_3). The positive ion FAB mass spectrum of **4** and the deoxy analog **7** yield strong protonated molecular ions and glycerol adduct ions allowing the direct and simple determination of molecular weight. Thus, positive ion FAB provides molecular weight identification of ribo- and deoxyribonucleosides not available using standard EI and CI conditions, and this technique should prove especially valuable in the mass spectral analysis of guanine and cytosine type nucleosides.

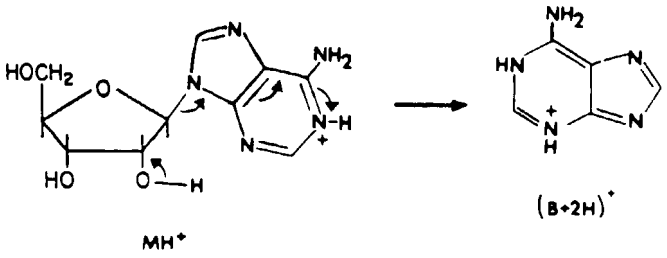
Cleavage of the glycosidic bond is the most prominent decomposition pathway observed in the mass spectra of nucleosides using either EI[25] or CI[27], and this fragmentation also produces the base peak in the spectra of nucleosides analyzed using positive ion FAB. Sission of the base-sugar bond with transfer of either one or two hydrogens from the carbohydrate to the aglycone[40] in EI yields both the $(B+H)^{+\cdot}$ and $(B+2H)^+$ ions, respectively. Structural correlations indicate the presence of a purine base or 2'-deoxy sugar favors formation of a more intense $(B+H)^{+\cdot}$ ion while the $(B+2H)^+$ ion prevails in the mass spectra of pyrimidines and some ribosides.[25] Ionization by CI or FAB, however, produces only the $(B+2H)^+$ ion. Thus, some structural information is lost relative to the EI spectrum since the type of base or sugar is not obvious from the intensity of only the $(B+2H)^+$ ion.

Fragmentations in the CI mode proceed from the protonated molecular ion MH^+ where the proton is derived from the reagent gas with the site of protonation, on the base or sugar, determining the subsequent route of fragmentation.[27] In a similar manner, decompositions in FAB proceed from the protonated molecular ion but, in this case, the proton is derived from the sample matrix, generally glycerol. Fragmentations in FAB may thus be more closely related to solution chemistry, and in fact, the potential utility of FAB measurements for the direct study of solution chemistry has previously been suggested.[41,42] However, despite differences in the source of the added proton, certain parallels between the FAB and CI spectra may be noted: (1) fragment ions in FAB are postulated to be the result of gas-phase unimolecular decomposition reactions arising from the protonated molecular ion,[8,43] and these gas-phase processes are envisioned as occurring just above the sample matrix surface;[35] (2) a

more elaborate ion-molecule charge-cascade concept has recently been proposed which seems to account for the CI-like nature of FAB spectra,[44] i.e., the obvious similarity, aside from intensity differences, between the even-electron ions present in FAB mass spectra and those ions observed in CI mass spectra using methane; (3) a correlation has been established between the solution hydrolysis of the glycosidic bond of nucleosides and the gas-phase cleavage, in the absence of solvent, of the base-sugar bond in the CI mode, that is, both liquid and gas-phase hydrolysis experiments yield the same qualitative result concerning glycosidic bond stability of nucleosides.[45] Therefore, the assumption is made that the fragmentation mechanisms proposed in CI[27] are generally applicable to decompositions observed in the positive ion FAB mass spectra.

Formation of the $(B+2H)^+$ ion in CI proceeds from the protonated molecular ion by transfer of a sugar hydroxyl hydrogen to the base, concomitant with glycosidic bond cleavage. The site of initial protonation, with the proton being derived from the reagent gas, is dependent on the aglycone but, according to solution hydrolysis, is presumably N1 for adenosine.[46] Equation 1 illustrates the mechanism for $(B+2H)^+$ ion production in CI for adenosine. The transfer of a sugar hydroxyl hydrogen and not a carbon bound hydrogen has been established and the use of deuterium labeled reagent gas[27] allows discrimination of reagent gas vs. OH hydrogens. Whether or not the same mechanism is occurring in FAB remains to be determined. Since FAB is similar to solution chemistry, the solvent matrix in FAB may be the source of all labile protons, as is normally observed in solution hydrolysis.[45] Furthermore, according to the ion-molecule charge-cascade phenomena mentioned above, the matrix, having the higher concentration, is ionized by collisions with the bombarding gas and the resulting charge is transferred to the sample upon collision with a sample molecule.[44] However, deuterium labeled glycerol cannot be used to confirm the mechanism since rapid H-D exchange occurs in this protic solvent[21] and discrimination of ribose OH vs. solvent hydrogens is lost.

Although the exact mechanism of formation is unclear, the $(B+2H)^+$ ion is the base peak in the FAB mass spectra of all nucleosides examined, except **9**. As shown in Table 1, analogous results are



Eq. 1 Formation of the $(B+2H)^+$ ion from MH^+ for adenosine in CI.[27] The same mechanism is thought to be operating in FAB.

obtained using CI (CH_4) and, in the case of the pyrimidine nucleosides, EI. The C-nucleoside, 9, shows a suppressed $(B+2H)^+$ ion in EI,[26] CI,[27] and FAB reflecting stronger C-C vs. C-N bond stability and thus, decreased glycosidic bond cleavage. Similar results have been observed using FAB MS/MS.[24]

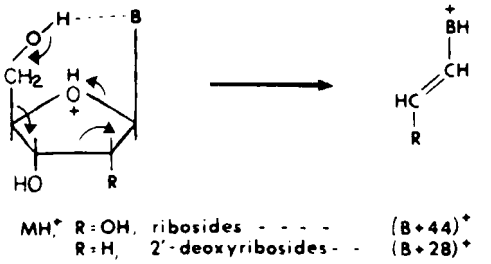
Although the FAB spectra of nucleosides are dominated by the MH^+ and $(B+2H)^+$ ions, other fragment ions of diagnostic value are also observed. A structurally useful ion generally present in the EI and CI mass spectra of both ribosides and 2'-deoxyribosides appears at M-89 ion (EI) or MH-90 (CI), and represents loss of carbons 3' through 5' with retention of a hydroxyl hydrogen.[25,27] This ion, commonly referred to as $(B+44)^+$ for ribosides and $(B+28)^+$ for 2'-deoxyribosides,[25] is a base-containing fragment important in determining the site of sugar modification in nucleosides of unknown structure. The assignment of this ion in an EI spectrum is unambiguous, but caution should be used in assigning a structure or relative intensity to this ion in the CI mode because of the formation of B + reagent gas adduct ions. For example, the use of isobutane gas in CI leads to a $(BH \cdot C_3H_7)^+$ adduct ion which occurs at the same nominal mass as the $(B+44)^+$ ion[27] of ribosides. In the FAB mass spectra, all of the nucleosides examined exhibited $(B+44)^+$ ions, analogous to the species observed in the CI spectra, except that reagent gas adducts are not present. Generally base-containing fragment ion intensities, such as $(B+44)^+$, are greater in EI than in CI due to the high-energy ionization in EI. In FAB, relative intensities of the $(B+44)^+$ ion are also less than those observed in EI, at least for the purine nucleosides. Relative ion abundance values of the $(B+44)^+$ ion for the pyrimidine

nucleosides, 3, 4 and 8, however, are within 2% of the intensities seen in EI.

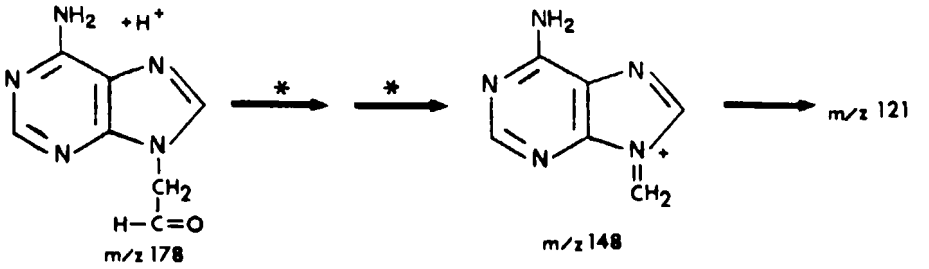
The mechanism for the formation of the $(B+44)^+$ ion in EI proceeds from the odd-electron molecular ion via radical-site directed cleavages, and differs in ribosides vs. deoxyribosides.[25] In contrast, the decomposition in CI originates from a protonated molecular ion and occurs by successive charge-site initiated cleavages, identical in the riboside and deoxyribosides, as indicated in Equation 2.[27] Although the site of initial protonation in CI is proposed to be the ether oxygen of the sugar,[27] fragmentation of the sugar in both EI and CI is initiated by abstraction of a sugar hydroxyl hydrogen by the base.[25,27]

Electron impact induced decomposition of the $(B+44)^+$ ion leads to even electron ions at m/z 148 and 121 in the mass spectrum of adenine nucleosides as shown in Eq. 3.[25] The first of these ions, the $(B+14)^+$ ion, is formed by expulsion of CHO· and H· radicals from the sugar while the m/z 121 is produced by elimination of HCN from the m/z 148 ion.[25] In FAB, only the isopropylidene derivative **10** displays a $(B+14)^+$ ion while both 1 and **10** show the m/z 121 peak with relative intensities of 3 and 4%, respectively.

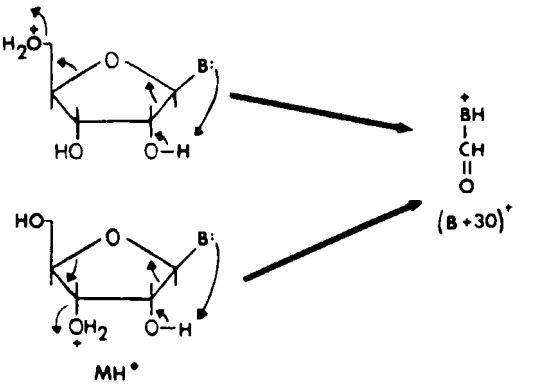
The $(B+30)^+$ ion provides information concerning modifications at the 1'- and 4'-positions of the sugar ring and confirms the assignment of the $(B+H)^{+\cdot}$ and $(B+2H)^+$ ions. This ion has been shown[25] to consist of the aglycone, the C-1' and O-4' positions of the carbohydrate ring and a hydrogen transferred to the base from the 2'-hydroxyl group. Decreased abundance of the $(B+30)^+$ ion is therefore observed in the EI spectra of 2'-deoxyribosides and other nucleosides lacking a 2'-OH group. The parents of this ion are the $M^{+\cdot}$ (EI) or MH^+ (CI) ions. The mechanism of formation of the $(B+30)^+$ ion in CI is similar to that proposed for EI except that heterolytic rather than homolytic bond cleavages occur. Fragmentation of the sugar with concomitant loss of water results in generation of the $(B+30)^+$ ion.[27] Essentially the same mechanism is thought to be operating in FAB and is illustrated in Equation 4. A particular advantage of FAB over CI in identification of the $(B+30)^+$ ion is the absence of potentially interfering reagent gas adduct ions. In the case where methane is the CI reagent gas, the $(BH\cdot C_2H_5)^+$ adduct ion falls at the same nominal mass as the $(B+30)^+$ ion.



Eq. 2. Formation of the $(B+44)^+$ and $(B+28)^+$ ions from MH^+ in CI.[27] Analogous processes are suggested to occur during FAB ionization.



Eq. 3 Decomposition of the $(B+44)^+$ ion in adenine nucleosides in EI.[25]



Eq. 4. Formation of the $(B+30)^+$ ion from MH^+ in CI.[27]

The $(B+30)^+$ ion is present in the spectra of all nucleosides examined using FAB ionization. As indicated in Table 1, the intensity of this ion is significantly reduced relative to the intensity observed with EI, and in some cases CI. The decreased intensity of the $(B+30)^+$ ion in FAB tends to lessen the contribution of this ion in confirming assignment of the $(B+2H)^+$ ion. The utility of this ion in detecting modifications at the 1'-and 4'-positions has not yet been examined with FAB but results comparable to CI may be anticipated in the analysis of, for example, 4'-thionucleosides.[47] Modifications at the 2' and 3'-positions, however, were differentiated with FAB MS/MS data using the $(B+30)^+$ ion in conjunction with the $(B+44)^+$ and $(B+2H)^+$ ions.[24]

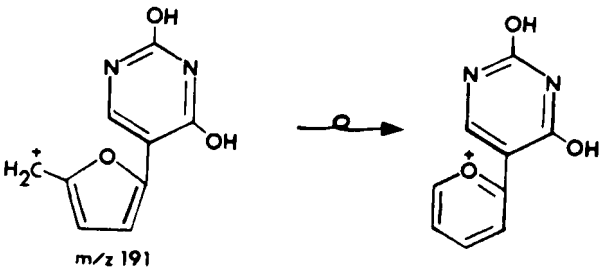
Analogous to EI, the FAB spectra of 2'-deoxyribosides displayed $(B+30)^+$ ions in decreased abundance compared to the corresponding ribosides. A more obvious distinction of the deoxy from the ribo analogs is, however, reflected in the relative intensity of the MH^+ and $(MH+G)^+$ where, in all cases, the deoxy series produce substantially less intense adduct ions. The relative intensity difference in these ions may be a reflection of the increased lability of the glycosidic bond of 2'-deoxynucleosides, relative to ribosides, toward acid hydrolysis. If indeed this is the case, rate constants for the acid catalyzed cleavage of the glycosidic bond of nucleosides should be available using FAB in much the same manner as has been described with CI.[40] Alternatively, the decreased intensity of the $(MH+G)^+$ ion may reflect a decrease in adduct forming ability of the deoxy vs ribo analogs, i.e., the presence of cis hydroxyls may permit formation of a more stable complex with the glycerol matrix.

Particularly interesting is the comparison of the EI, CI and FAB spectra of pseudouridine (9) where significant differences are observed depending on the mode of ionization. The $(B+30)^+$ ion as the base peak in the EI spectrum of a nucleoside has generally been considered to be diagnostic of a C-nucleoside,[48] although the possibility of exceptions to this "rule" have been suggested.[49] Pseudouridine, a naturally occurring constituent of t-RNA[50] and a typical C-nucleoside, thus displays the $(B+30)^+$ ion as the base peak in the EI spectrum.[25] The base peak in the CI spectrum depends on the reagent gas but, in the methane spectrum, is the $(B+44)^+$ ion at m/z

155.[47] The $(B+30)^+$ ion in the CI (CH_4) spectrum is considerably diminished in intensity relative to the EI mode but is still significant, appearing at m/z 141 with a relative intensity of 19%.[27] Fast atom bombardment ionization of 9 produces a spectrum dominated by the MH^+ ion which is accompanied by a fairly substantial $(MH+G)^+$ ion at m/z 337 (17%). The $(B+30)^+$ ion, however, is relatively insignificant (m/z 141, 8%) in the FAB spectrum, and the $(B+44)^+$ ion, although present as the second most intense peak, is of only 29% relative intensity.

The obvious change in base peak with ionization mode probably reflects either differences in excess energy imparted to the sample during ionization (EI significantly greater) or variation in the site of protonation. With methane as the reagent gas in CI, either the sugar or aglycone may undergo protonation with subsequent fragmentations reflecting the "mixed" parents. On the other hand, FAB appears to protonate sites on the heterocyclic base to a much greater extent than sites on the sugar. An example of the preferred protonation sites in CI vs. FAB is the series of ions related to the elimination of $x \cdot H_2O$, where x = 1, 2 or 3 molecules of water from the MH^+ ion. While differences in the EI and CI spectra (relative to FAB) may be attributed to changes in the probe temperature or heating rate, the loss of $x \cdot H_2O$ molecules observed in the FAB spectrum most likely represent true fragmentations since the spectrum was obtained without probe heating. The most abundant ion of this series, in all three ionization modes, is the $(MH-2H_2O)^+$ or $(M-2H_2O)^{+\cdot}$ peak. The $(MH-2H_2O)^+$ ion at m/z 209 is, however, of significantly greater intensity in the CI spectrum (54%) compared to the EI (6%) and FAB (7%) modes. Whatever the cause for this change - thermal effects, differences in site of protonation, the energy content of precursor ions, or the presence of solvent in the FAB mode - the difference is striking.

Decomposition of m/z 209 in CI and FAB may occur by at least two possible pathways. The loss of an additional water molecule from m/z 209 gives a highly conjugated fragment ion at m/z 191 that may undergo a ring expansion reaction to produce an aromatic pyrilium ion, indicated in Equation 5, similar to the well-documented tropilium ion.[51] Relative ion abundances of the m/z 191 ion are 4% and 1% in CI and FAB, respectively.

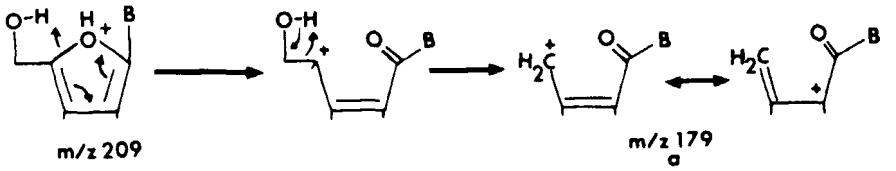


Eq. 5. Proposed rearrangement reaction for m/z 191 in **9** with CI and FAB.

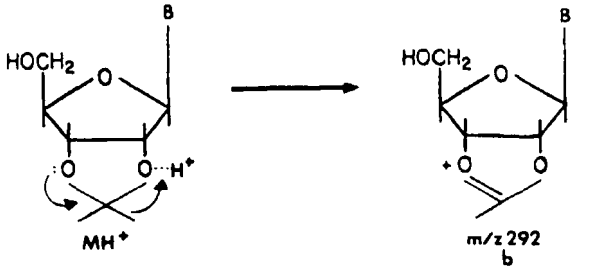
A second fragmentation pathway available to the m/z 209 ion is loss of formaldehyde to form ion **a** at m/z 179 ion as shown in Equation 6. Intensities of ion **a**, 20% in CI and 8% in FAB, are significantly increased over those observed for m/z 191, suggesting that the second pathway is the preferred route for decomposition of m/z 209. High resolution and metastable ion studies will be necessary to confirm this proposal.

The FAB mass spectrum of the 2',3'-O-isopropylidene compound **10** demonstrates the analysis of a volatile derivative of a nucleoside. Many of the important fragments indicative of the isopropylidene functionality in EI[25] are also present in the FAB mass spectrum of **10**. As with free nucleosides, the major fragmentation of **10** is glycosidic cleavage producing the $(B+H)^{+\cdot}$ and $(B+2H)^{+}$ ions in EI. Similarly, FAB analysis affords the $(B+2H)^{+}$ ion as the base peak in the spectrum. Loss of a methyl radical from the isopropylidene function of the $M^{+\cdot}$ ion in EI generates a highly stabilized tertiary carbonium ion, designated ion **b**, at m/z 292.[25] A low intensity ion of the same nominal mass is present in the FAB spectrum, representing loss of a molecule of methane from the MH^{+} ion, as shown in Equation 7.

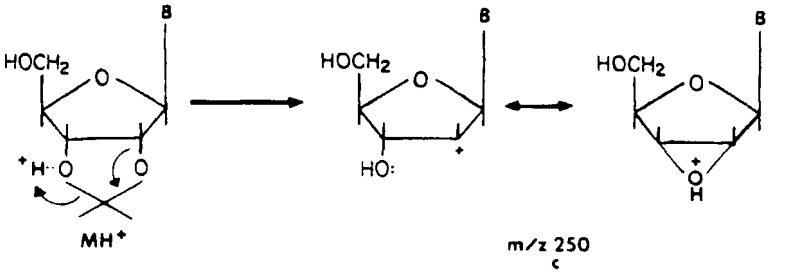
The loss of acetone from the isopropylidene function of the molecular ion related species is observed in FAB and a similar loss is evident in the EI spectrum. The radical ion **c** appearing at m/z 249 in EI is depicted structurally as a 2',3'-epoxide.[25] In FAB, the corresponding loss of acetone from the protonated molecular ion yields m/z 250, an ion which probably exists as the protonated 2',3'-epoxide, consistent with the observation of predominantly even electron ions in



Eq. 6. Proposed formation of ion **a** at m/z 179 from m/z 209 for **9** in CI and FAB.



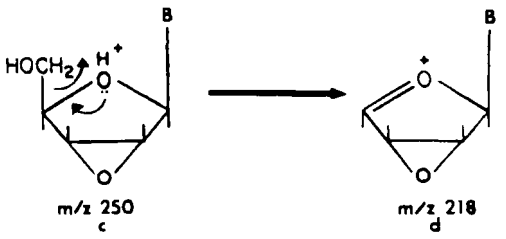
Eq. 7. Proposed formation of ion **b** at m/z 292 from MH^+ for **10** in FAB.



Eq. 8. Proposed mechanism of formation of ion **c** at m/z 250 from MH^+ for **10** in FAB.

FAB. A mechanism for the formation of ion **c** in FAB is proposed in Equation 8.

Further elimination of the 5'-hydroxymethylene group from m/z 249 by simple radical site directed cleavage and established for **10** by metastable ion analysis in EI, affords an abundant ion **d** at m/z 218.[24] Ion **d** also appears in the FAB mass spectrum of **10** at m/z 218, but at a much lower intensity than that seen in EI. The decreased intensity may be attributed to the occurrence of a proton transfer reaction in FAB



Eq. 9. Proposed decomposition of ion **c** to form ion **d** at m/z 218 for **10** in FAB.

resulting in the loss of methanol to generate ion **d**, depicted in Equation 9, rather than simple cleavage as in EI.

The low intensity of fragments indicative of the isopropylidene function observed in the FAB spectrum of **10** obtained using glycerol as the matrix prompted the investigation of sulfolane (tetramethylene sulfone, MW 120) as an alternative solvent. The use of a second solvent also serves to verify that the ions associated with the fragmentation of **10** are, in fact, sample related and not artifacts produced from the glycerol. The use of sulfolane as a solvent for **10** results in an increase in the relative strength of the FAB spectrum *vs.* that obtained in glycerol. An enhanced solubility of **10** in sulfolane may explain the increased intensity of all ions observed in the FAB spectrum using this solvent *vs.* glycerol. Although identical conditions were used when sulfolane was the matrix, application of additional solvent was necessary during data acquisition because of the increased volatility of sulfolane relative to glycerol. Adduct ion formation of sulfolane (SF) with the MH^+ ion, designated as $(MH+SF)^+$, occurs analogous to the $(MH+G)^+$ ion with glycerol, and the $(MH+SF)^+$ ion appears at m/z 428 for **10**. When compared to the EI spectrum of **10**, the sulfolane spectrum shows a two fold increase in relative intensity for at least two key fragments, the $(B+30)^+$ and S^+ ions.

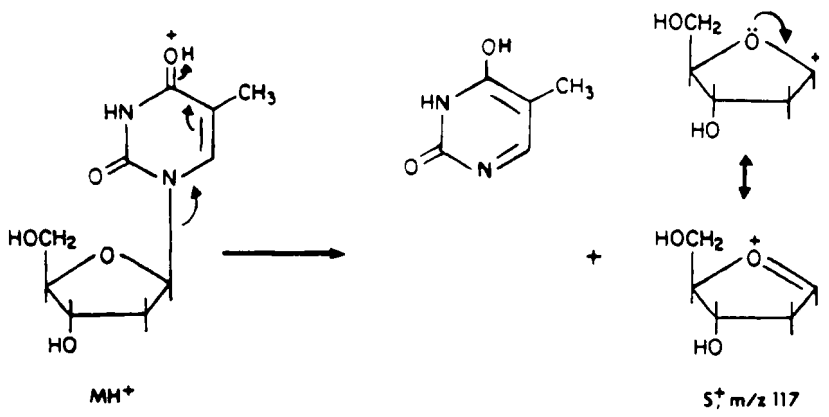
The most distinguishing feature of the FAB spectrum of **10**, however, is not the presence of certain base fragments but, more importantly, a very prominent MH^+ ion (53% in glycerol and 64% in sulfolane). In contrast, the molecular ion of **10** in EI is of significantly lower intensity (2%), despite the enhanced volatility

provided by the isopropylidene function. Analysis of the isopropylidene riboside serves to illustrate the utility of FAB in examining derivatives of nucleosides which may be necessary intermediates in the synthesis of new riboside analogs.

Other base-containing fragments in EI, e.g., $(M-CH_2O)^{+\cdot}$, a structural indicator of the 5'-functionality, the $(B+60)^+$ ion, a riboside peak consisting of C-1' and C-2' along with the base,[25] and the $(B+41)^+$ ion, an indicator of cytidine analogs,[39] are absent in CI and FAB mass spectra of all nucleosides examined. Therefore, some structural information is not available by these modes of ionization which is present in the EI spectrum.

Fragments indicative of purine base decomposition, as established in EI based using metastable anaylses, deuterium labels and analog comparison, are also apparent with FAB. Many of these ions are similarly found in CI, but their presence has not been discussed in previous literature. Only ions occurring above m/z 100 are examined in the present work in order to avoid the extremely intense m/z 93 ion of glycerol. Also, since FAB characteristically produces ions at every mass,[2,3] care must be taken in interpreting low mass and low intensity ions.

Expulsion of HCN from the $(B+2H)^+$ ion produces a peak at m/z 109 in the FAB mass spectra of the adenosine analogs **1**, **5** and **10**, which corresponds to the same neutral loss from the $(B+H)^{+\cdot}$ ion in the EI mode.[25] Guanosine analogs, on the other hand, demonstrate at least three base decomposition pathways in EI. The usual loss of the amino function as NH_3 in EI may occur in FAB yielding m/z 135 in **2** and **6** with relative intensities of 4% and 2%, respectively. Another prominent EI fragment in the cleavage of the guanine base is elimination of cyanamide. The FAB mass spectra of **2** and **6** indicate an ion at m/z 110 which corresponds to this loss from the $(B+2H)^+$ ion. Expulsion of HNCO constitutes a last base decomposition route for guanine observed in EI and an appropriate ion is also observed with FAB. Fragmentation proceeds from the $(B+H)^{+\cdot}$ ion in EI giving rise to m/z 108, while m/z 109 is generated in FAB from the $(B+2H)^+$ ion. In each of the three above mentioned fragments, relative ion intensities of the riboside as compared to the corresponding deoxy derivative are essentially the same.



Eq. 10. Proposed formation of sugar ion, S^+, from MH^+ for **8** in FAB.

Retention of charge by the ribose moiety following glycosidic bond rupture results in the formation of sugar ions by many ionization modes, including FAB. Generally decreased abundance of sugar ions of the purine nucleosides is observed in EI and CI, resulting from preferential protonation of the base in the electron-rich purines.[25,27] The trend of increased sugar ion abundance in pyrimidine nucleosides is also observed in FAB, especially in the mass spectra of uridine and thymidine where S^+ ions, (m/z 133 (11%) in **3** and (m/z 117 (38%) in **8**, are evident. A plausible mechanism for the formation of S^+ ions in CI involves sugar protonation leading to glycosidic bond cleavage with concomitant loss of a base molecule.[27] Again, the FAB mechanism is probably somewhat analogous to that proposed for CI, although the proposed site of protonation may be more appropriately assigned to the base, as indicated in Equation 10 for thymidine.

Suppression of ions related to glycosidic bond rupture has already been established for C-nucleosides,[39] like **9**, in EI[25] and CI.[27] However, the FAB mass spectrum of **9** exhibits a relatively intense ion (16%) corresponding to $(S-H_2O)^+$ at m/z 115. Since this mass is coincident with a glycerol plus sodium adduct, the exact composition of the m/z 115 ion must be confirmed by high resolution. In the case of **10**, the sugar ion characteristic of isopropylidene derivatives, occurs at m/z 173 and is abundant only in the uridine

derivative in EI.[25] The FAB mass spectrum of **10** also demonstrates a weak sugar ion at m/z 173 (1.3% RI), which is easily distinguishable from background noise and is also present in the spectrum using sulfolane (3% RI).

Negative Ions

Apparatus for operation of FAB in the negative ion mode is commonly installed in conjunction with the FAB source and voltage supply for positive ions. Therefore, unlike EI which requires extensive instrumental modification,[52] minor adjustments make feasible the interchange of positive to negative ion detection in a matter of seconds. Sensitivity in the negative ion mode, however, is diminished as compared to the positive ion mode due to repulsion of the negative ions by a conventional secondary electron multiplier operating at increased gain. Modifications to the SEM to overcome this reduced sensitivity have been described.[53] The preliminary results described below are therefore not optimized because of the current limitations of the instrument. Negative ion CI data was not included in the following discussion since no reports on its use in the analysis of nucleosides have appeared in the literature.

Determination of the molecular weight of a nucleoside in the negative ion FAB mass spectra is based on the presence of a strong $(M-H)^-$ ion. The $(M-H)^-$ ion falls 2d below the MH^+ ion in the positive ion FAB spectra and 1d below the $M^{+\cdot}$ ion in positive ion EI spectra, thus yielding complementary molecular weight information. Although relative ion intensities in negative ion EI spectra are present at a 1-10% level,[52] relative ion intensities of the molecular ion species of nucleosides in the negative ion FAB spectra are typically above 10% (See Table 2). In some cases, such as deoxyguanosine (**6**), the $(M-H)^-$ ion in the negative ion spectrum is over 20% higher (66%) than the MH^+ ion observed in the positive ion spectrum (43% RI). Adenosine, on the other hand, shows a much less intense $(M-H)^-$ ion (12% RI) in the negative ion mode, possibly owing to differences in basicity of the aglycones.

The number of fragmentation pathways observed in the negative ion FAB spectra are decreased considerably compared to the positive ion

spectra, a situation similar to the positive/negative relationship in EI.[52] Utilization of a few prominent ions indicative of molecular weight of the nucleoside and the free base can, however, aid in characterization of these structural moieties, and thus complement the more detailed fragmentation obtained in the positive ion mode.

The primary fragmentation route in the negative FAB and EI mass spectra is production of the base ion, B^-, following cleavage of the glycosidic bond.[52] With the exception of pseudouridine and dihydrouridine, the B^- ion is the most intense peak in the spectra of all free nucleosides examined via negative ion FAB or EI. This observation is attributed to extensive charge delocalization in the bases of the pyrimidine and purine type.[52] Assignment of the molecular weight of the base directly from the negative ion mass spectrum is, therefore, possible using either FAB or EI. However, when compared to the corresponding positive ion spectra, FAB offers the distinct advantage that the base fragments B^- and $(B+2H)^+$ are the most intense peaks (100%) in both detection modes. Consequently, the obvious difference of 2d between the intense base fragments in the positive and negative ion FAB spectra serves to establish the identity of the base fragment simply and quickly.

Sugar ions, corresponding to S^+ and $(S-H_2O)^+$ in the positive ion mode, are not observed to any appreciable degree in the negative ion mass spectra using FAB or EI.[52] Selective charge retention on the base, rather than the sugar, is a result of stabilization by charge delocalization and may explain the absence of sugar ions in the negative ion mode.

A diagnostic fragment analogous to the $(B+44)^+$ ion in the positive ion mode is the $(B+42)^-$ ion. Data from isotopic labeling of uridine in negative ion EI indicates that the $(B+42)^-$ contains two carbons and the 2' oxygen of the sugar,[52] analogous to the $(B+44)^+$ ion in the positive ion spectra. Ion intensities of this $(B+42)^-$ ion in the deoxyribosides are significantly decreased or absent in both the negative ion EI[52] and FAB mass spectra. Although relative intensities of the $(B+42)^-$ ion in the corresponding ribosides are nearly identical using EI or FAB, mechanisms for the formation of this ion from the molecular ion species are not identical due to the lack of radical ion generation in the FAB technique. Generally, the pyrimidine ribosides

exhibit a more intense $(B+42)^-$ ion than the purines, possibly due to the increased basicity in the purines which leads directly to formation of the stabilized B^- ion.

Another major fragmentation pathway in the negative ion EI or FAB mass spectra of adenosine is the loss of ammonia from the B^- ion yielding m/z 117.[52] Although this ion is also formed from N^6-methylated adenosine analogs by loss of methylamine in the negative ion EI mode,[52] only adenosine was examined in FAB. The intensity of the m/z 117 ion for adenosine in FAB is of the same magnitude (10%) as observed in EI (12%).

Although absent from the negative ion EI mass spectrum,[52] uridine displays an ion corresponding to the loss of 43d from the $(M-H)^-$ ion in the negative ion FAB spectrum. Presumably formed by a Retro-Diels-Alder mechanism with elimination of HNCO, the m/z 200 ion is observed in the negative ion FAB spectrum with a relative intensity of 7%. Similarly, the MS/MS negative ion FAB spectra of uridine and 4-thiouridine[24] exhibit very intense ions consistent with the loss of 43d. from $(M-H)^-$. Since the loss of HNCO was previously observed for pyrimidines only in positive ion EI, the presence of this ion in negative ion FAB may represent an additional structural feature indicated by this ionization mode.

Conclusions:

The utility of positive ion FAB mass spectrometry for the analysis of nucleosides is successfully demonstrated on the eight major ribo- and 2'-deoxyribonucleosides found in DNA and RNA, and two related analogs. A number of distinct advantages are illustrated by FAB as compared to the standard modes of EI and CI and to other desorption ionization methods.

The production of intense MH^+ ions in the spectra of all nucleosides examined, including the more thermally labile guanosine and cytidine analogs, is a major advantage of FAB. The MH^+ ion along with glycerol adducts of the form $(MH+Gn)^+$ simplify molecular weight determination. The $(B+2H)^+$ ion, generated by glycosidic cleavage, is the base peak in all FAB spectra except for the C-nucleoside, pseudouridine. In addition, a number of structurally diagnostic fragment ions, e.g., $(B+30)^+$, $(B+44)^+$ and S^+, are observed with FAB.

Mechanistic correlations between CI and FAB are obvious due to the predominance of even electron ions, among other factors, formed by these two techniques. However, the production of certain base decomposition fragments, established in EI and suspected in FAB, may be an obvious advantage over CI.

Another distinct feature not previously mentioned is ability of FAB to analyze volatile derivatives, such as the 2',3'-O-isopropylidene ribosides. Structurally useful ions produced by FAB for this class of derivative are consistent with those ions observed in EI, with the exception that a much more intense molecular ion species is produced with FAB.

The analysis of nucleosides by negative ion FAB offers structural information complementary to that obtained from positive ion spectra. Negative ion FAB spectra are simple, exhibiting intense molecular ions of the type $(M-H)^-$, and some fragment ions, i.e., $(B+42)^-$ and B^- ions. Consequently, molecular weight determination is facilitated by comparison of the negative ion with the positive ion spectra. Off setting the advantage of simplified spectra in the negative ion mode is the loss in sensitivity and some structural information.

Minimal instrument adaptation for FAB ionization, simple sample preparation and reproducible results are also attractive features inherent to the FAB technique. The general utility of FAB in the mass spectral analysis of highly polar, thermally labile compounds makes analysis via FAB of prime significance for samples not amenable to EI or CI.

A major disadvantage of FAB, however, is the presence of artifact peaks arising from the matrix. The problem of matrix interferences may be circumvented by either using MS/MS,[24] whereby matrix effects are totally eliminated, or by using different matrix systems and comparing resulting mass spectra. In the latter case, a data system can be used to subtract interfering matrix peaks from each solvent used without the danger of eliminating important sample peaks.

In conclusion, the FAB technique of mass spectrometry is a valuable tool in the analysis of nucleosides, yielding structural information comparable to EI and CI, and protonated molecular ions of higher abundance than observed by these standard methods.

Acknowledgements:

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