for conventional procedures, our experiments with CK-MM and CK-BB should be sufficient to ensure the applicability of the procedure described herein to the quantitation of the B subunit in CK-MB. Furthermore, although we do not have detailed quantitative data for CK-MB, we have done preliminary experiments to show that the anti-CK-MM inhibits 50% of the CK-MB activity as expected. Accordingly, although our objective was not to develop a working clinical procedure, but rather to evaluate a new approach, results strongly suggest that the new approach can be developed into a practical method.

ACKNOWLEDGMENT

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Registry No. Creatine kinase, 9001-15-4.

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Fast Atom Bombardment and Tandem Mass Spectrometry of Covalently Modified Nucleosides and Nucleotides: Adducts of Pyrrolizidine Alkaloid Metabolites

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Twelve different modified nucleoside and nucleotide adducts containing guanosine, adenosine, uridine, or thymidine bonded to various pyrrolizidine alkaloid metabolites were investigated by using fast atom bombardment (FAB) and tandem mass spectrometry (MS/MS). The goal is to compare and understand the collisionally activated decompositions of gas-phase $(M - H)^-$, $(M + H)^+$, and $(M + K)^+$ ions, where M is the modified nucleoside or nucleotide. The $(M - H)^-$ ions of nucleoside adducts fragment simply to lose the sugar moiety whereas the $(M - H)^-$ of the nucleotides decompose by losing either the sugar or the alkaloid and by forming the nucleic acid base as B^-. The abundances of the base-containing ions correlate with the known stabilities of the bases. Decomposition of the $(M + H)^+$ leads to liberation of the alkaloid as an electrophile. The protonated nucleic acid base (BH_2^+) and the chemically modified base are also formed, and their abundances correlate with the proton affinities of the bases. $(M + K)^+$ ions decompose differently than the $(M + H)^+$ to give principally ions containing the base moiety and potassium. K^+ appears to be principally affiliated with the base or nucleoside moieties, indicating that these structural units have high potassium ion affinities. The proton, on the other hand, is more closely associated with the base and/or the modified base. The fragmentation reactions provide information relating to the molecular weight of the covalently bonded alkaloid and to the nature and sites of modification of the base, sugar, and alkaloid.

The detection and structure elucidation of covalently modified nucleosides and nucleotides from various genetic biopolymers are important in a variety of modern research areas, particularly biochemistry and oncology. One challenge to the chemist is to develop suitable information-rich methods that are applicable to highly polar, thermally sensitive materials and that can be applied to small (microgram or less) quantities isolated from living systems.

The advent of fast atom bombardment (FAB) (*1*, *2*) has provided the mass spectrometrist with such a technique. The successful application of FAB to the analysis of a variety of nonderivatized nucleosides, nucleotides, and modified nucleosides and nucleotides has been reviewed recently (*3–7*). There are several potential drawbacks in the application of FAB/MS analyses. One is that there is often little fragmentation information which could be used for structure determination. The use of a chemical matrix can pose difficulties in that low abundance fragment ions can be obscured by the chemical noise due to the matrix. As many of the compounds of biochemical interest exist as mixtures, assignment of parent–daughter relationships, when fragment ions are observed, can be quite difficult.

Tandem mass spectrometry (MS/MS) (*8*) is a technique that, when combined with FAB, can overcome the often-encountered drawbacks of the use of FAB alone. With this technique, an ion of interest is selected and induced to decompose by collisional activation and a spectrum is taken of the fragments (called an MS/MS or CAD spectrum). In this

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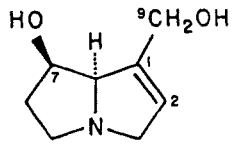
way, chemical noise can be largely eliminated, nonfragmenting ions induced to fragment, and parent–daughter relationships determined when mixtures are present.

FAB combined with MS/MS has been applied successfully to the determination of nucleosides and nucleotides, as well as to some modified nucleosides and nucleotides (*9–17*).

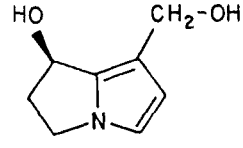
In this paper, we evaluate the application of FAB combined with tandem mass spectrometry for the purpose of determining the structures of an extensive series of nucleoside and nucleotide adducts. The method is illustrated by an investigation of a group of adducts of pyrrolizidine alkaloid metabolites. This study is of a sufficiently complete series of nucleoside and nucleotide adducts so as to permit general conclusions to be drawn on the modes of decomposition of these and related materials in the gas phase.

Pyrrolizidine alkaloids are found in a wide variety of plant species of the genera *Senecio, Crotalaria, Heliotropium, Erechtites, Trichodesma*, and *Amsinckia*. These plants are widely distributed and are of particular concern to livestock owners because of their hepatotoxicity (*19, 20*). In recent years, these plants have also become recognized as potentially hazardous to man.

Most naturally occurring pyrrolizidine alkaloids are retronecine (structure I) mono- or diesters or dilactones derived from the dicarboxylic necic acids (*21*). An important structural feature required for toxicity is the presence of a 1,2 double bond. The toxic action of these alkaloids, however, is thought to be exerted through reactive pyrrole derivatives such as dehydroretronecine (DHR, structure II) (*22*) or its esters or dilactones.



I



II

Pyrrolizidine alkaloids have been detected in honey produced by bees pollinating tansy ragwort (*Senecio jacobaea L.*) flowers (*23, 24*) and in milk from cattle (*25, 26*) and goats (*27*) that ingested ragwort. These alkaloids are carcinogens (*28, 29*) and mutagens (*30*), whose pyrrolic metabolites are known to react with DNA (*31*) and other cellular nucleophiles (*32, 33*). Recently, however, the toxic effect was reported to be associated with a novel metabolite, 4-hydroxy-2-hexenal (*34*).

Adducts of DHR and all of the mononucleosides and mononucleotides, except those of cytosine, were isolated and characterized (*9*). The reactive C7 site of DHR was shown to alkylate N6 of adenosine, O2 of thymidine (thymine-2-deoxyriboside), O2 of thymidine 5′-monophosphate, and N2 of deoxyguanosine 5′-monophosphate. By analogy it was inferred that alkylation occurred at O2 of uridine and of uridine 5′-monophosphate (*9*).

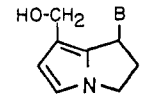
EXPERIMENTAL SECTION

Instrumentation. MS/MS spectra were obtained with a Kratos MS-50 triple analyzer tandem mass spectrometer, which was previously described (*35*). This instrument consists of a high-resolution MS-I of Nier-Johnson geometry followed by an electrostatic analyzer used as MS-II. Samples were dissolved in a minimum amount of methanol, and a 1-μL aliquot was added to the matrix (acidified glycerol or dithiothreitol/dithioerythritol for the acquisition of positive ion mass spectra and triethanolamine or glycerol for negative ion spectra). Because of the unusual nature of these compounds, macroscopic amounts of each sample were not available. We can only estimate that quantities in the range of 1–5 μg were required for each investigation. Fast atom bombardment by 7-keV xenon atoms was used to desorb the preformed ions from the matrix, which was supported on a copper probe held at +8 kV (positive ion mode) or −8 kV (negative ion mode).

MS/MS or collisionally activated decomposition (CAD) spectra were obtained by activating the ions in the third field free region by collision with helium gas (sufficient to suppress the ion beam by 50%) and by scanning MS-II. Detection was with a postacceleration detector at 15 kV with respect to ground. Ten to thirty scans were signal averaged for each spectrum by using software developed at the Midwest Center for Mass Spectrometry.

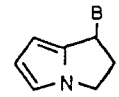
Table I. Compounds Investigated

Dehydroretronecine Adducts



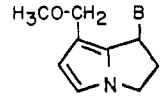
1a	B = guanosine
2a	B = adenosine
3a	B = thymidine
4a	B = uridine
5a	B = thymidine 5-monophosphate
6a	B = deoxyguanosine 5′-monophosphate

Dihydropyrrolizidinol Adducts



1b	B = guanosine
2b	B = adenosine
3b	B = thymidine
4b	B = uridine

Methyl Ethers of Dehydroretronecine Adducts



1c	B = guanosine
3c	B = thymidine

Materials and Methods. Retronecine was prepared by hydrolyzing monocrotaline (*36*). Dehydroretronecine was synthesized by dehydrogenating retronecine with chloranil (*37*). The crude product was purified by subliming it and crystallizing it from hexane–acetone. Nucleotides (free acid form) and nucleosides were obtained from Sigma Chemical Co.

The monofunctional 2,3-dihydro-1*H*-pyrrolizin-1-ol was prepared by reduction of the ketone (200 mg) with sodium borohydride (47 mg) in water (15 mL). The reaction was complete in 4 h. The reaction mixture was extracted three times with an equal volume of diethyl ether, and the combined ethereal extracts were dried over sodium sulfate, filtered, and evaporated to give 190 mg of a white crystalline material. Final purification by sublimation (35 °C, 0.025 mmHg) gave 152 mg of product, mp 66–67 °C [lit. (*38*), oil]. NMR and MS data were consistent with published data (*39, 40*). The starting ketone was prepared by cyanoethylation of pyrrole (*38*) and subsequent cyclization of the intermediate nitrile (*40*).

Preparation of Adducts. The preparation of nucleoside and nucleotide adducts of DHR was described previously (*9*). The adducts investigated in this study are listed in Table I, and their structures are presented in Figure 1. Methyl ethers **1c** and **3c** were produced by reaction of the adducts with the methanolic eluent during isolation by HPLC.

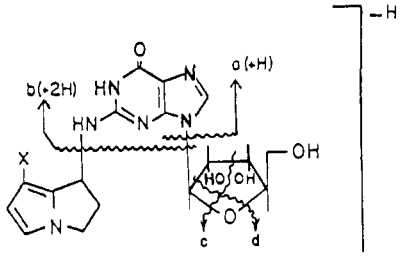
RESULTS AND DISCUSSION

Nomenclature. In the following discussion we will use the abbreviated nomenclature given in Table II for the species discussed in this paper.

CAD Spectra of (M − H) Anions. *Nucleoside Adducts.* The CAD spectra of the (M − H) anions of the nucleoside adducts are dominated by an ion, $(RB - 1)^-$, resulting from loss of the sugar residue (see Figure 2A for example). Less abundant fragments, B^- and those formed by cleavages of the sugar ring, denoted as S_1 and S_2, are also observed (*15*). The B^- ion arises by loss of the sugar and the dehydroretronecine (X = CH_2OH) or the dihydropyrrolizinol (X = H) moieties with hydrogen transfer (Scheme I and Table III). The sugar fragmentations are relatively more facile for compounds containing the purine bases than for those having the pyrimidine bases.

Nucleotide Adducts. The CAD spectra of the dehydroretronecine deoxyguanosine 5′-monophosphate adduct, **6a**, and

Scheme I



X = H, CH_2OH

path a → $(RB - 1)^-$
path b → B^-
path c → $S_1(-C_3H_6O_3)$
path d → $S_2(-C_4H_8O_3)$

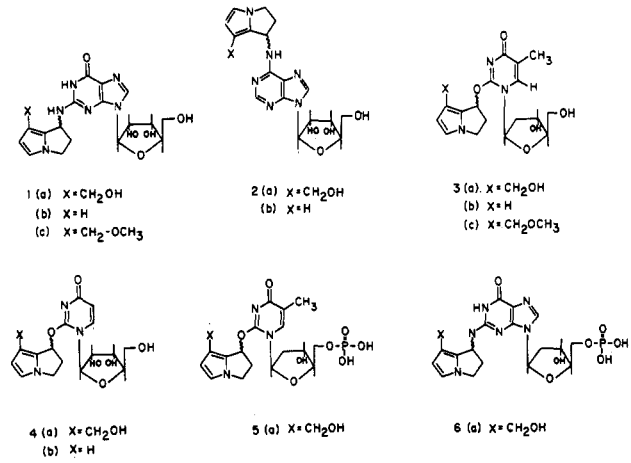
Table II. Shorthand Nomenclature for Species Discussed in the Paper[a]

neutral	cation	anion
RH[b]	R^+	
BH[c]	BH_2^+, BHK^+	B^-
RB[d]	RBH^+, RBK^+	$(RB - 1)^-$
SH[e]	SH_2^+	
BS[f]	BSH^+, BSK^+	$(BS - 1)^-$
$B(S - 1)PO_3H_2$[g]	$B(S - 1)PO_3H_3^+$, $B(S - 1)PO_3H_2K^+$	$B(S - 1)PO_3H^-$
$(RBS - 1)$[h]	$(RBS - 1)H^+$, $(RBS - 1)K^+$	$(RBS - 1 - H)^-$
M[i]	$(M + H)^+$, $(M + K)^+$	$(M - H)^-$
	$(S - 2)PO_3H_3$, $(S - 2)PO_3H_2K^+$	$(S - 2)PO_3H^-$
	S_1(loss of $C_3H_6O_3$)	S_1(loss of $C_3H_6O_3$)
	S_2(loss of $C_4H_8O_3$)	S_2(loss of $C_4H_8O_3$)

[a] The use of −1 and −2 in the shorthand nomenclature is for bookkeeping purposes and does not relate to the mechanism of ion formation; i.e., $(RB - 1)^-$ does not arise from proton abstraction from RB. [b] RH = neutral alkaloid or modifying species. [c] BH = neutral base. [d] RB = neutral modified base. [e] SH = neutral sugar. [f] BS = neutral nucleoside. [g] $B(S - 1)PO_3H_2$ = neutral nucleotide. [h] $(RBS - 1)$ = neutral modified nucleoside. [i] M = neutral molecule.

Table III. Relative Abundances of Ions in the Negative Ion FAB MS/MS Spectra of Nucleoside Adducts

	$(M - H - H_2O)^-$	S_1	S_2	$(RB - 1)^-$	B^-	other
1a	3	14	4	100	15	$RB - 1 - H_2O$ (10%)
2a	1	3	1.5	100	3	$(B - H_2O)^-$ (1%); (M − H − 44) 22%
3a	<1	1		100	1	$RB - 1 - H_2O$ (1%)
4a		1	<0.5	100	1	M − H − 43 (<1%)
1b	3	15	9	100	7	
2b	2	3	<1	100	1	
3b	<1			100	<1	
4b	<1	2	2	100	1	
1c	10	15	5	100	6	$(RB - 1 - H_2O)^-$ = 20%
3c	70	20		100	4	$(RB - 1)$ less CH_2 = 20%; $(M - H - CH_3OH)^-$ = 70%



Nucleoside and Nucleotide Adducts of Dehydroretronecine, Dehydroretronecine methyl ether, and Dihydropyrrolizinol

Figure 1. Structures of adducts studied.

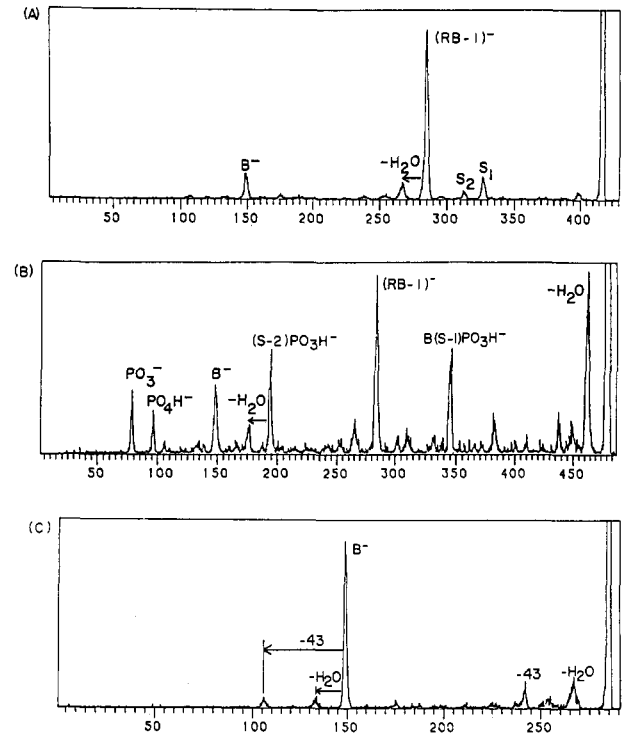


Figure 2. (A) CAD spectrum of the $(M - H)^-$ of the dehydroretronecine guanosine adduct. (B) CAD spectrum of the $(M - H)^-$ of the dehydroretronecine deoxyguanosine 5′-monophosphate adduct. (C) CAD spectrum of the $(RB - 1)^-$ ion of dehydroretronecine deoxyguanosine 5′-monophosphate adduct.

the thymidine 5′-monophosphate adduct, **5a**, are significantly more informative than the CAD spectra of the nucleosides (e.g., see Figure 2B). Ions representative of all portions of the molecule are apparent (Scheme II and Table IV).

The ion designated as $B(S - 1)PO_3H^-$ results from cleavage of the retronecine–base bond with hydrogen transfer to the base. Ion $(RB - 1)^-$ results from cleavage of the base–sugar bond. Ion B^- represents the base and arises via cleavage of

Table IV. Relative Abundances of Ions in the Negative Ion FAB MS/MS Spectra of Nucleotide Adducts

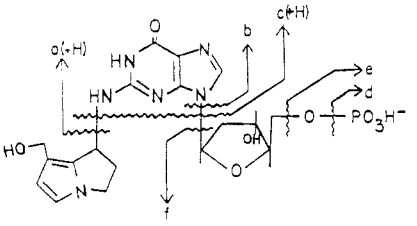
compound	$-H_2O$	$-H_3PO_4$	$B(S - 1)PO_3H^-$	$(RB - 1)^-$	$(RB - 1 - H_2O)^-$	$(S - 2)PO_3H^-$	$[(S - 2)PO_3H - H_2O]^-$	B^-	PO_4H^-	PO_3^-	other
5a	11	1	42	100		97	9	3	12	10	m/z 330 = 6
6a	100	21	66	96	17	65	14	38	23	35	

Table V. Relative Abundances of Ions in the FAB MS/MS Spectra of $(RB - 1)^-$ Ions

compound	$-H_2O$	B^-	other identified ions
1a	18	100	$(RB - 1 - 43) = 19\%$; $(B - 16)^- = 4\%$; $(B - 43)^- = 4\%$
2a	8	100	$(B - 16)^- = 6\%$; $(B - HCN)^- = 7\%$
3a	18	100	$(B - 43)^- = 3\%$; m/z 42 = 6%; $(RB - 1 - CH_2O)^- = 11\%$; $(RB - 1 - CH_3OH)^- = 17\%$
4a	15	100	$(B - 43)^- = 7\%$; m/z 42 = 9%; $(RB - 1 - CH_3OH) = 25\%$
5a	16	100	$(B - 43)^- = 5\%$; m/z 42 = 8%; $(RB - 1 - CH_2) = 9\%$; $(RB - 1 - CH_3OH)^- = 13\%$
6a	18	100	$(RB - 1 - 43)^- = 15\%$; $(B - 43)^- = 6\%$; $(B - 16)^- = 6\%$
1b	*a*	100	$(RB - 1 - 43)^- = 6\%$;[a] $(B - 43)^- = 10\%$[a]
2b	*a*	100	$(B - 43) = 50\%$; $(B - 16) = 8\%$

[a] Spectrum contaminated with background ions.

Scheme II



path a → $B(S - 1)PO_3H^-$
path b → $(RB - 1)^-$
path c → B^-
path d → PO_3^-
path e → PO_4H^-
path f → $(S - 2)PO_3H^-$

both the base–sugar and base–retronecine bonds with transfer of one hydrogen. Ion $(S - 2)PO_3H^-$ (Scheme II) results from scission of the base–sugar bond and transfer of one hydrogen to the departing neutral base. The formation of the ion via path f may be viewed as a 1,2-elimination occurring remote from the charge site (*41*).

Consideration of the masses of ions produced via paths a, b, c, and f provides information as to which subunits (i.e., alkaloid, base, or sugar) of the nucleotide have been modified.

The relative abundances of the $(RB - 1)^-$ and B^- ions reflect the known relative stabilities of the base anions (*42–45*). Thus, the collisionally activated (CA) decompositions of the purine-containing adduct, which is more stable than an adduct with a pyrimidine base, yield $(RB - 1)^-$ as the most abundant fragment and an abundant B^- ion. On the other hand, the CA decompositions of the pyrimidine adduct give little B^-, and the $(RB - 1)^-$ ion is comparable in abundance to the $(S - 2)PO_3H^-$ anion (see Table IV).

CAD Spectra of the $(RB - 1)^-$ Anion. The CAD spectra of the $(RB - 1)^-$ ions are dominated by abundant B^- ions (Table V). The guanosine adducts, **1a** and **6a**, fragment by losing 43 mass units (CHNO) from the purine ring (see as an example Figure 2C). A similar loss was observed in the CAD spectra of thymidine and uridine and shown to arise via NHCO loss from the pyrmidine ring (*15*). A similar mechanism is expected to be operating in the guanosine case; i.e., loss of NHCO from the six-membered ring of the purine moiety. If this is true, then this fragmentation can be used as evidence for the site of attachment of the alkaloid to the base; i.e., the site cannot be O6 or N1. The thymidine, **3a–c**, and uridine, **4a,b**, adducts do not undergo this loss, which is a major fragmentation route of the nucleosides themselves (*15*). The absence of ions arising by loss of 43 mass units in the spectra of the thymidine and uridine adducts is consistent with the assigned structure in which O2 is now blocked. However, after the alkaloid moiety is lost, the resulting B^- ion undergoes the expected loss of NHCO. These conclusions must be approached with some caution since conclusions can be misleading when they are based on the absence of an expected fragmentation.

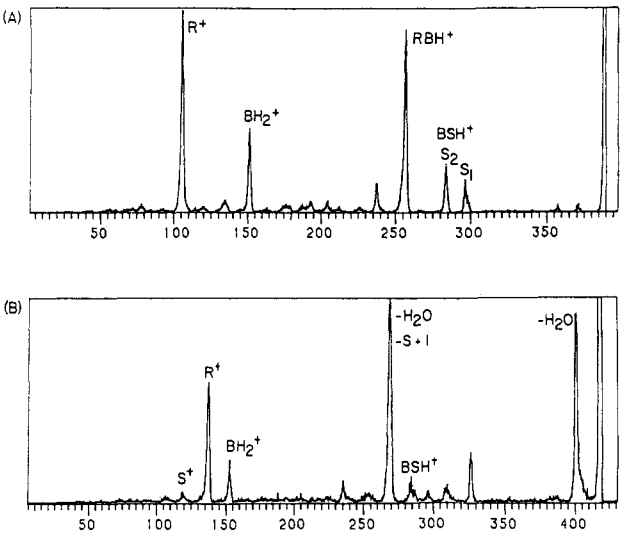
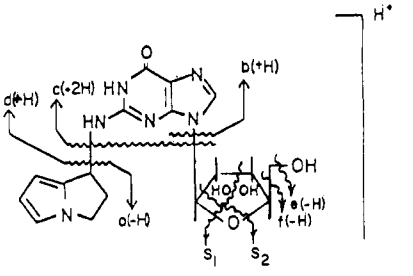


Figure 3. (A) CAD spectrum of the $(M + H)^+$ of the dihydropyrrolozinyl guanosine adduct. (B) CAD spectrum of the $(M + H)^+$ of the dehydroretronecine guanosine adduct.

Scheme III



path a → $R^+(m/z\ 106)$
path b → RBH^+
path c → BH_2^+
path d → BSH^+
path e → $(M + H - H_2O)^+$
path f → $(M + H - CH_3OH)^+$

CAD Spectra of $(M + H)^+$ Ions. *Dihydropyrrolizinol Adducts.* The CA decompositions of the $(M + H)^+$ ions of dihydropyrrolizinol adducts lead to an ion of m/z 106 (designated as R^+), which is the most abundant fragment for all the adducts studied (see Figure 3A as an example). Additional major fragment ions are the protonated base (BH_2^+), the protonated modified base (RBH^+), and the protonated nucleoside (BSH^+) (Scheme III). These latter three ions are formed in competition with R^+, and as a result, some conclusions about relative proton affinities can be drawn by comparing the relative abundances of BH_2^+, RBH^+, and BSH^+ with that of R^+ (see Table VI).

The sums of the abundances of all the base-containing ions relative to R^+ are greater for the modified purines (guanosine and adenine) than for the pyrimidines. This is consistent with

Table VI. Relative Abundances of Ions in the FAB MS/MS Spectra of $(M + H)^+$ Ions of Nucleoside Adducts

compound	M + H − H_2O	$-CH_3OH$	S_1	S_2	RBH^+	cyclic $-H_2O-S+1$	BSH^+	R^+	BH_2^+	S^+	other
1a	93		1	1	6	100	13	59	21	9[a]	$(-H_2O - S + 1 - 16) = 3\%$
2a	100	1	2	3	40	24	42	45	9	4[a]	
3a	100	4	2		7		9	18	4	6[a]	$BSH^+ - C_4H_5NO = 4\%$ m/z 105 = 9%
4a	100	7	3	4	19	5	20	45	3	5[a]	$(R^+ - CH_2OH)^+ = 11\%$
1b	3	3	6	*b*	95		23	100	43	2	m/z 238 = 15%
2b	5	2	5		23		98	100	25	5	
3b	60	4			7		60	100	20	5	
4b	17	3			5		13	100			
1c	55	100	4	3	7	75[c]	18	5 37 (Me)	13	10	S_1 from $[MH - CH_3OH] = 20\%$ m/z 221 = 25%
3c	47	24	10		100		18	37	6	20	$BSH^+ - C_4H_5NO = 10\%$ $RO{-}C{\equiv}N^+ = 14\%$ m/z 238 = 15%

[a] Overlaps R^+. [b] Overlaps BSH^+. [c] As $(M + H - CH_3OH - S + 1)^+$.

the proton affinities of the bases and the nucleosides in the gas phase (B, A > T, U) as determined by Wilson and McCloskey (*46*). Moreover, all $(M + H)^+$ ions of the modified nucleosides, except of the modified guanosine, fragment to give more abundant protonated nucleosides (BSH^+) than RBH^+ and BH_2^+, which indicates that the proton affinities of the nucleosides are greater than those of the modified bases. Wilson and McCloskey also found that gas-phase nucleosides are more basic than their respective bases (*46*).

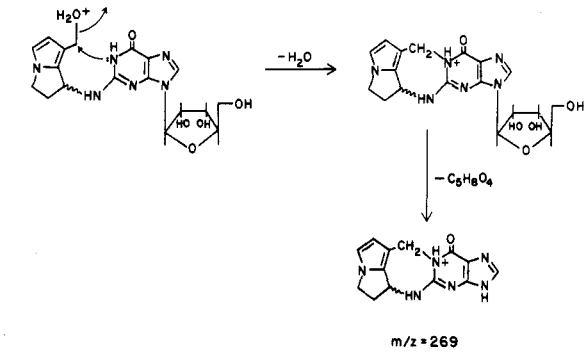
The abundance of the modified base (RBH^+) for the guanosine adduct is greater than the BSH^+ and BH_2^+ abundances. The modified guanine apparently is more basic (has a higher proton affinity) than the nucleoside guanosine. We have no explanation for this reversal.

Dehydroretronecine Adducts. The difference between dehydroretronecine adducts and those discussed above is that hydroxymethylene ($-CH_2OH$) substituent on the necine base ring of the former substances. The $-CH_2OH$ substituent has a marked effect on the fragmentation of the $(M + H)^+$ ions of the modified nucleosides. The $(M + H)^+$ ions of all the adducts undergo abundant loss of water (see **1a–4a** in Table VI). Moreover, the $(M + H - H_2O)^+$ ions of the modified guanosine, adenosine, and uridine fragment further by expelling the sugar moiety accompanied by hydrogen rearrangement to the base. For example, these products are the ions of m/z 401 and 269, respectively, in the CAD spectrum of the modified guanosine (see Figure 3B). There is no evidence for the loss of H_2O and sugar (presumably sequential) from adducts that do not contain the $-CH_2OH$ substituent (see Table V, entries for **1b–4b**). The activation barrier for production of the ion of m/z 269 from $(M + H)^+$ of modified guanosine must be lower than the barriers for R^+ and RBH^+, BSH^+, and BH_2^+ as these latter ions are no longer abundant (see Figure 3B and Table VI, entry for **1a**).

A fragmentation scheme that is consistent with the results involves assisted water loss by the nitrogen lone pair either of the pyrimidine bases or of the six-membered ring of the purine bases. As a result, a cyclic ion is formed (see Scheme IV). The assistance by the base must be considered important so that differences in the relative abundances of the cyclic ions can be understood. We would expect that the guanosine ring could best stablize the positive charge, and both processes are most facile for this modified nucleoside. Following water loss, the sugar moiety is eliminated with H-transfer back to the base, possibly as a remote-charge-site fragmentation (*41*).

The CA decompositions of the dehydroretronecine–guanosine adduct methyl ether, **1c**, are similar. Loss of H_2O from the $(M + H)^+$ ion is reduced in abundance while a facile loss of CH_3OH occurs. The cyclic ion of m/z 269 remains unaltered in mass, but the mass of the dehydroretronecinyl cation is shifted 14 amu to m/z 136. The implication of these data for structure elucidation is that the site of alkylation of **1c** is readily located (in this case the dehydroretronecinyl oxygen).

Scheme IV



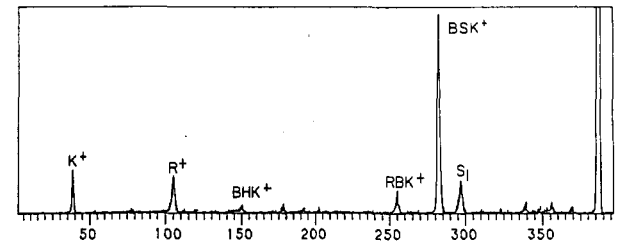


Figure 4. CAD spectrum of the $(M + K)^+$ of the dihydropyrrolozinyl uridine adduct.

CAD Spectra of $(M + K)^+$ Ions. *Dihydropyrrolizinol Adducts.* The decomposition reactions of the $(M + K)^+$ ions of dihydropyrrolizinol adducts (Figure 4 and Table VII) are dominated by loss of the pyrrole with proton transfer to yield the $(M + K)^+$ ion of the parent nucleoside, BSK^+. Other significant ions include S_1^+, formed by sugar cleavage [the ion S_2^+ is obscured by BSK^+], RBK^+, BHK^+, and K^+ (Scheme V).

It is noteworthy that the most facile decomposition of the $(M + H)^+$ always leads to R^+ whereas the $(M + K)^+$ ions primarily fragment to give products with the K^+ associated with base (BSK^+, RBK^+, and BHK^+). The R^+ ions are still formed, but they are of lower relative abundance than those formed from the $(M + H)^+$ species. Therefore, the potassium ion affinities of the bases, the modified bases, and the nucleosides appear to be greater than their respective proton affinities.

Dehydroretronecine Adducts: The $(M + K)^+$ ions of the dehydroretronecine adducts fragment in a manner similar to

Table VII. Relative Abundances of Ions in the FAB MS/MS Spectra of $(M + K)^+$ Ions

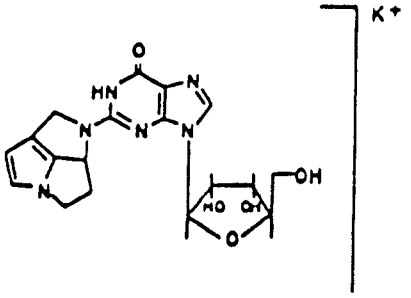
compound	$(MK - H_2O)^+$	S_1	S_2	BSK^+	RBK^+	BHK^+	R^+	$(M + K - H_2O - S + 1)$	K^+	other
1a	100	7	10	32	13	5	6	7	3	m/z 273/278 = 6% $(M + K - H_2O - S + 1)^+$ = 5%
2a	97	44		58	100	15	19	21	50	$(M + K - CH_3OH)$ = 35%
3a	10	13	5	100	26	7	16		14	
4a	4	10	9	100	<15[a]		23	4	15	
1b	15	*b*	*c*	100	30	20	30		38	$(BSK - 16)^+$ = 9%
2b	*b*	*b*	*b*	80	100	25	40		*b*	
3b	7	31		100	17	9	25		32	$M + K - CH_3OH - S + 1$ = 28%
4b	3	16		100	12	4	19		22	$(M + K - CH_3OH)^+$ = 5% $(M + K - 49)$ = 6%
1c	13	26	7	4[d] 18	7	3	3[d]	7	3	$(M + K - CH_3OH)^+$ = 100% m/z 289 = 4% m/z 349 = 4
3c	20			18[d] 100	35	14	10 45[d]	*c*	59	$(M + K - CH_3OH)^+$ = 15%

[a] Overlaps BSK^+. [b] Background prevents accurate measurement. [c] Same mass as $M + K - H_2O - S + 1$. [d] Methyl ether.

that of the corresponding dihydropyrrolizine adducts (Table VII); i.e., S_1^+, S_2^+, BSK^+, RBK^+, BHK^+, R^+, and K^+ are formed. However, unlike the dihydropyrrolizine adducts, the dehydroretronecine $(M + K)^+$ ions containing adenosine and guanosine undergo rather facile water elimination reactions. The fragmentation involving losses of water and a sugar species are significantly less favorable. The guanosine adduct is noteworthy: the protonated adduct undergoes loss of water and $C_5H_8O_4$ to give the most abundant fragment of m/z 269, whereas the m/z 307 fragment, in which the proton is replaced by K^+, is less than 10% relative abundance (see Table VII).

The loss of water is only a minor reaction channel for the $(M + K)^+$ ions of the modified pyrimidine nucleoside **3a** and **4a**. In contrast, it is a major fragmentation for the $(M + H)^+$ ions of both the pyrimidine and purine nucleosides. Nevertheless, the loss must involve the -OH of the $-CH_2OH$ substituent of the alkaloid as water loss is nearly nonexistent for the dihydropyrrolizine $(M + K)^+$ ions. Because the fragmenting ion contains K^+ in lieu of H^+, the elimination of water must involve H transfer from another site. Both modified purine nucleosides contain an NH group connecting the alkaloid and base moieties whereas the corresponding group for the two modified pyrimidines studied here is an oxygen atom without an available hydrogen atom.

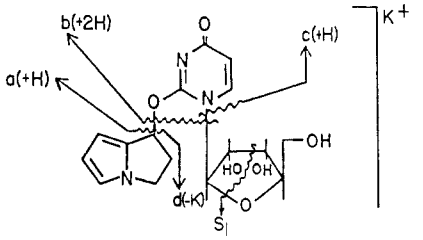
The mechanism for the water expulsion can be pictured as a 1,5-elimination involving the groups discussed above to give the product ion, **7**, which contains a new five-membered ring.



7

We view the K^+ to be situated principally with the base–sugar part of the molecule. If it were associated with the dihydropyrrolizinyl $-CH_2OH$, the loss of KOH might be expected, but this loss is not observed. Because the K^+ is located remote from the reaction site, the reaction for water loss is considered to be a thermal process, not charge-induced (*41*). Little if any sugar moiety ($C_5H_8O_4$) loss with H transfer follows the water elimination because that part of the molecule is associated with the K^+ and prefers to remain charged.

Scheme V



path $S_1 \rightarrow MK^+ - C_3H_6O_3$
path a → BSK^+
path b → BHK^+
path c → RBK^+
path d → R^+

Table VIII. Relative Abundance of Loss of R vs. Loss of S from $(M + K)^+$ Ions of Nucleoside Adducts

compound	base	$RBK^+(-S + 1)$	$BSK^+(-R + 1)$
2a	adenosine	100	50
1a	guanosine	50	100
3a	thymidine	30	100
3b	thymidine	18	100
4a	uridine	10	100
4b	uridine	12	100

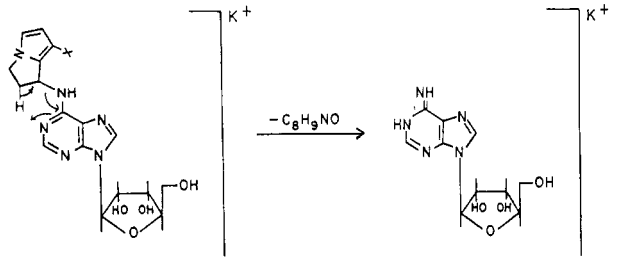
The difference in chemistry exhibited by the $(M + H)^+$ and the $(M + K)^+$ ions can be understood by invoking both charge-driven and charge-remote fragmentations. The loss of water from the $(M + H)^+$ of the dehydroretronecine-modified nucleosides is viewed as a charge-assisted process. The hydroxyl function to be eliminated may not be the site of highest proton affinity; nevertheless, those higher internal energy forms of $(M + H)^+$, which are protonated at the site, will undergo rapid water loss. The subsequent elimination of the sugar moiety ($C_5H_8O_4$) (with H transfer) occurs remote from the charge site as the charge of the $(M + H - H_2O)^+$ is now maintained on a very stable quaternary nitrogen. However, the $(M + K)^+$ ion undergoes a thermal loss of water, which occurs remote from the site of cationization. For $(M + K - H_2O)^+$, loss of the sugar moiety ($C_5H_8O_4$) would be proximate to the charge site, and the reaction does not occur.

Another feature of the fragmentation chemistry of the $(M + K)^+$ ions is the smooth decrease in the RBK^+ abundance relative to the BSK^+ abundance as the modified nucleoside is changed from adenosine to guanosine, thymidine, and/or uridine (Table VIII). This ordering is also manifested by

Table IX. Relative Abundances of Ions in the FAB MS/MS Spectra of Nucleotide Adduct $(M + K)^+$ Ions

compound	$-H_2O$	$B(S-1)-PO_3H_2K^+$	S_1	$-HPO_4$	$-H_2O-PO_4H$	R^+	K^+	BHK^+	$(S-2)-PO_3H_2K^+$	RBK^+	$(RBK-H_2O)^+$
5a	100	6	1	0.5		1.2	0.5		0.3	1	0.5
6a	>100	100	22	12	5	27	25	28	24	85	87

Scheme VI



dinucleotide $(M - H)^-$ ions in their fragmentations to lose BH from the 3′ position (*16*). Loss of adenosine as BH is favored over the loss of guanosine as BH, and these losses of purine bases are preferred relative to pyrimidine base loss. However, for the present example, the phenomenon is a result of a competition of the nucleoside and the modified base for the potassium ion. For all cases except the modified adenosine, the formation of the potassiated nucleoside is favored, consistent with the K^+ preferring to be coordinated with the nucleoside moiety of the molecule. Thus, the potassium ion affinity of the nucleoside is higher than that of the modified base. For adenosine, on the other hand, the loss of retronecine with H transfer to give BSK^+ involves substantial loss of aromaticity of the adenosine base, and the loss of the sugar to yield RBK^+ now becomes competitive (see Scheme VI). Formation of BSK^+ is not mitigated by loss of aromaticity for any of the other modified nucleosides. The transition state in Scheme VI is speculative and is intended only to illustrate the loss of aromaticity and not the origin of the transferred hydrogen.

The CAD spectra of the $(M + K)^+$ ions of the methyl ethers of guanosine, **1c**, and thymidine, **3c**, adducts were also obtained. On the basis of the spectrum of the guanosine methyl ether adduct, **1c**, it is clearly confirmed that the retronecine hydroxyl function has been methylated. The BSK^+ and BHK^+ ion masses remain unchanged while the R^+ and RBK^+ ion masses have shifted up 14 mass units. Instead of abundant loss of H_2O, loss of CH_3OH is observed. This is expected in accord with the proposed mechanism for H_2O loss from the $(M + K)^+$ ions.

The CAD spectrum of the $(M + K)^+$ ion of the thymidine methyl ether, **3c**, adduct is more complicated. It is likely from the masses of R^+ and $(R + 14)^+$, $(M + K - H_2O)^+$, and $(M + K - CH_3OH)^+$, and BSK^+ and $(BSK + 14)^+$ that this modified nucleoside exists as a mixture containing either the dehydroretronecinyl hydroxyl (major component) or the nucleoside (minor component) in a methylated form. The relatively low abundance of the BHK^+ ion and the overlap of the S + 14 and R^+ preclude determining whether the position of methylation is the base or the sugar.

Dehydroretronecine Nucleotide Adducts. The CAD spectra of the $(M + K)^+$ ions of the dehydroretronecine thymidine 5′-monophosphate, **5a**, and the dehydroretronecine deoxyguanosine 5′-monophosphate, **6a** (Figure 5), adducts are presented in Table IX. The major fragment ion arises from expulsion of H_2O. As has been observed for other thymidine adducts, transfer of H from R and loss of (R – H) to give the cationized unmodified nucleotide is a prominent process. Upon collisional activation of the $(M + K)^+$ ion of the deoxyguanosine nucleotide, formation of ions arising from loss

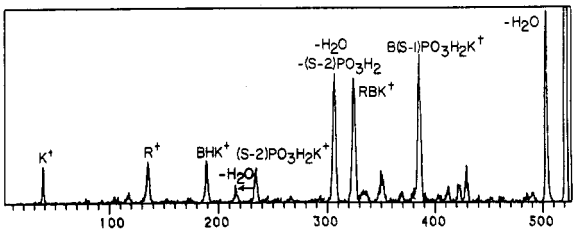


Figure 5. CAD spectrum of the $(M + K)^+$ of the dehydroretronecine deoxyguanosine 5′-monophosphate adduct.

of HPO_4 and from loss of HPO_4 and H_2O compete with loss of (R – H). Expulsion of RB to yield an ion of m/z 235 is also noted. This fragmentation occurs for the $(M + K)^+$ of the thymidine nucleotide, **5a**, but to a lesser extent. The contrast in behavior of the modified pyrimidine nucleotide (R–B bond breakage strongly dominating) and the modified purine nucleotide (R–B bond cleavage less dominant) is consistent with the differences observed for the fragmentations of the $(M + H)^+$ ions containing these bases. An abundant ion of m/z 269 is also formed in the decomposition of the $(M + H)^+$ of the deoxyguanosine 5′-monophosphate adduct.

CONCLUSION

The combination of fast atom bombardment and tandem mass spectrometry provides useful information for the structure determination of electrophile–nucleoside and –nucleotide adducts. Collisional activation causes these biomolecules to come apart to form their principal building blocks. Sites of base, sugar, and alkaloid modification can be identified and in some cases information as to the site of alkaloid–base bonding is present. The most informative decompositions are those of the $(M + H)^+$ and $(M + K)^+$ ions. The fragmentation of the $(M - H)^-$ ions of the nucleotides is also highly useful, but the CAD spectra of the $(M - H)^-$ ions consist primarily of $(RB - 1)^-$ and provide only confirmatory information. Some minor fragment ions from the $(M - H)^-$ are indicative of the site of alkaloid base bonding, however.

The CAD spectra provide, in addition to structural information, an opportunity for comparing the reactivity of the four bases and contrasting the behavior of purine- and pyrimidine-containing modified nucleosides and nucleotides. The contrast in reactivity between the $(M + H)^+$ and $(M + K)^+$ ions of the dihydroretronecine adducts depends on the possible sites of cationization by the proton and potassium ion and on the role of charge in fragmentation of complex biomolecules.

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Microwave Energy for Acid Decomposition at Elevated Temperatures and Pressures Using Biological and Botanical Samples

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A closed vessel microwave digestion system is described. In situ measurement of elevated temperatures and pressures in closed Teflon PFA vessels during acid decomposition of organic samples is demonstrated. Temperature profiles for the acid decomposition of biological and botanical SRMs are modeled by the dissolving acid. Microwave power absorption of nitric, hydrofluoric, sulfuric, and hydrochloric acids is compared. An equation is applied to acid microwave interactions to predict the time needed to reach target temperatures during sample dissolution. Reaction control techniques and safety precautions are recommended.

Sample preparation is a critical step in chemical analysis. It frequently establishes the lower limit in an elemental analysis through its influence on the analytical blank. Acid decompositions that are necessary prior to instrumental trace element analysis are time-consuming and are usually the slowest step in the analysis. Whereas phenomenal advances have been made in analytical instrumentation, sample preparation methods have not changed significantly in recent years. Indeed, the impetus to prepare large numbers of samples in less time and with greater efficiency is fostered by multielemental instruments that analyze samples in a fraction of the time needed to prepare them.

The use of microwave energy as the heat source in acid digestion was first demonstrated a decade ago (*1*, *2*). Since that time there have been several papers describing specific applications to open beaker acid digestions where significant time savings have been demonstrated (*3-7*). Several studies have compared the technique with different digestion procedures and have applied it successfully to a variety of different samples (*8-10*). Open vessel work leads to corrosion of equipment and risks environmental contamination of the

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