salt. After being stirred at 0 °C for 3 h, the reaction mixture was slowly warmed to room temperature and was stirred for an additional 2 h. It was then poured onto 20 mL of saturated NH_4Cl solution and extracted with CH_2Cl_2 (3 × 25 mL). Solvent was removed from the dried (Na_2SO_4) extracts under reduced pressure, and the residue was chromatographed on two silica gel preparative-layer plates, which were eluted with 3% acetone/EtOAc. The band with R_f 0.47 was cut out and eluted with 6% acetone/EtOAc. The dienaminone **17** was isolated as a yellow oil: 0.035 g (17% yield); UV max (EtOH) 388 nm (ε 43 346); ^1H NMR ($CDCl_3$) δ 1.26 (t, 3 H, J = 7.3 Hz), 2.92 (s, 6 H), 3.47 (s, 2 H), 4.13 (q, 2 H, J = 7.3 Hz), 5.15 (t, 1 H, J = 12.1, 14.5 Hz), 5.86 (d, 1 H, = 14.5 Hz), 6.76 (d, 1 H, J = 12.1 Hz), 7.34 (dd, 1 H, J = 14.5, 12.1 Hz).

Anal. Calcd for $C_{11}H_{17}NO_3$: C, 62.53; H, 8.11; N, 6.63. Found: C, 62.25; H, 7.84; 6.34.

Dienaminones of 3-Methoxy-2-cyclopentenone, 18a,b. The 3-methoxy-2-cyclopentenone was prepared by the method of House and Rasmusson.[34] The reaction residue, an amber oil, was purified by sublimation at 50–55 °C (bath temperature; 0.5 torr). The product, a white solid, was isolated in 81% yield: mp 49–51 °C (lit.[34] mp 51–52 °C); mass spectrum, m/z (relative intensity) 112 (M^+, 100), 83 (M^+ – CHO, 44), 81 (M^+ – OCH_3, 41), 69 (M^+ – C_2H_3O, 97), 57 (38).

The reaction of the vinamidinium salt 1 with 3-methoxy-2-cyclopentenone was carried out by method D. The residue after workup was chromatographed on aluminum oxide preparative-layer plates which were developed with 10% acetone/EtOAc. Two compounds were isolated, **18a** and **18b**, as yellow oils.

The band with R_f 0.40 was removed and eluted with 2% CH_3OH/EtOAc. The dienaminone **18b** was isolated as a yellow oil: 12% yield; UV max (EtOH) 395 nm (ε 45 400); ^1H NMR ($CDCl_3$) δ 2.85 (s, 6 H), 2.96 (m, 2 H), 3.86 (s, 3 H), 4.85 (t, 1 H, J = 12.1 Hz), 5.33 (s, 1 H), 6.51 (d, 1 H, J = 12.1 Hz), 6.60 (d, 1 H, J = 12.1 Hz); mass spectrum, m/z (relative intensity) 193 (M^+, 11), 178 (M^+ – CH_3, 7), 167 (M^+ – C_2H_2, 29), 149 (M^+ – $N(CH_3)_2$, 88), 97 (21), 70 (60), 43 (100).

The band with R_f 0.30 was cut out and eluted with 2% MeOH/EtOAc. The dienaminone **18a** was isolated as a yellow oil: 19% yield; UV max (EtOH) 395 nm (ε 45 431); ^1H NMR ($CDCl_3$) δ 2.88 (s, 6 H), 3.12 (m, 2 H), 3.82 (s, 3 H), 4.87 (t, 1 H, J = 12.1 Hz), 5.41 (s, 1 H), 6.66 (d, 1 H, J = 12.1 Hz), 7.02 (d, 1 H, J = 12.1 Hz); mass spectrum, m/z (relative intensity) 193 (M^+, 10), 178 (M^+ – CH_3, 4), 167 (M^+ – C_2H_2, 32), 149 (M^+ – $N(CH_3)_2$, 100), 97 (6), 70 (10), 43 (10).

Both of these compounds were too unstable to give satisfactory elemental analysis. However, mass, UV, and NMR spectral data provided excellent confirmation of the structure in each case.

Acknowledgment is made to the NSF for Grant No. CHE 79-058865 and to the University of Iowa Graduate College for a Research Assistantship to C.S.C.

Registry No. (*E*)-1, 70669-77-1; (*E*)-**2**, 78804-79-2; (*E,E*)-**3**, 75834-03-6; (*Z,E*)-**4a**, 78804-80-5; (*E,E*)-**4b**, 78804-81-6; (*E,E*)-**5a**, 75847-94-8; (*E,E*)-**5b**, 78804-82-7; (*E,E*)-**6**, 78804-83-8; (*E,E*)-**7**, 78804-84-9; (*E,E*)-**9a**, 75833-99-7; (*E,E*)-**10**, 75834-00-3; (*E,E*)-**11**, 78804-85-0; (*E,E*)-**12**, 75834-01-4; (*E,E*)-**13**, 78804-86-1; (*E,E*)-**14**, 78804-87-2; (*E,E*)-**15**, 75834-05-8; (±)-(*E,E*)-**16**, 78804-88-3; (*E,E*)-**17**, 78804-89-4; (*E,E*)-**18a**, 78804-90-7; (*Z,E*)-**18b**, 78804-91-8; β-(dimethylamino)acrolein, 927-63-9; dimethylamine·HCl, 506-59-2; diethyl malonate, 105-53-3; ethyl acetoacetate, 141-97-9; 3-pentanone, 96-22-0; 2-butanone, 78-93-3; cyclopentanone, 120-92-3; cyclohexanone, 108-94-1; cycloheptanone, 502-42-1; *dl*-camphor, 21368-68-3; estrone 3-methyl ether, 1624-62-0; γ-butyrolactone, 96-48-0; δ-valerolactone, 542-28-9; 1-ethyl-2-pyrrolidinone, 2687-91-4; 2-ethyl-2-oxazoline, 10431-98-8; 2-picoline, 109-06-8; 3-methoxy-2-cyclopentenone, 4683-50-5.

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Identification of Modified Nucleosides by Secondary-Ion Mass Spectrometry

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Argon ion bombardment of nucleosides produces mass spectra of high quality from which the molecular weight and the nature of the sugar can readily be determined. The most intense peak corresponds to the base itself, and ions representing the base attached to Na^+, or other cations, are obtained by addition of the appropriate salt. Parallels with fragmentations observed in electron impact exist and help to define molecular structures. Detection limits of a few nanograms are demonstrated and it is shown that ions characterizing the base can be greatly enhanced by simple acid pretreatment of the sample. Comparisons are made with pyrolysis mass spectrometry/mass spectrometry and it is concluded that modified bases cannot be identified in intact DNA by pyrolysis MS/MS without the possibility of isomerization although MS/MS does provide isomer specificity for pure modified bases.

The characterization of alkylated nucleosides at submicrogram levels has become increasingly important to nucleic acids and cancer research since most alkylating agents possess mutagenic activity and it is now recognized that most, if not all, chemical carcinogens are mutagens.[1,2] Nucleic acids generally have been considered prime target molecules for mutagenic and carcinogenic agents.[3,4] This paper deals with the capabilities of one of the newer ionization methods in mass spectrometry, secondary-ion mass spectrometry (SIMS),[5,6] for identification and quantification of methylated nucleosides. The companion paper[7]

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Table I. Comparison of the SIMS and PDMS Spectral Data of 7-Methylguanosine

secondary-ion mass spectrometry						
m/z	149	166	188[a]	208[a]	296	298
	10	100	1-3	2-5	0.3	0.3
plasma-desorption mass spectrum[b]						
m/z	149	166	188	208	296	298
	6	100	3	5	0	1

[a] Ion intensity varies somewhat between samples and with with surface concentration. [b] Data from ref 22.

applies this analytical method, in concert with nuclear magnetic resonance, liquid chromatography and ^{14}C-tracer studies, to determine the degree of methylation induced in a homopolynucleotide on treatment with methyl methanesulfonate.

Mass spectrometry has already played a vital role in delineating the structures of various modified nucleosides in transfer RNA,[8] viral and bacterial DNA,[9] and eukaryotic DNA.[10] Electron-impact mass spectra of underivatized purine and pyrimidine bases yield the molecular ion as the base peak along with diagnostic fragment ions. Similarly, isomers of methylguanine[11] and methyladenosine[12] can be distinguished by their electron-impact mass spectra. Chemical-ionization mass spectra are more useful for the more complex nucleosides which exhibit the protonated molecule, the protonated base generated by glycosidic cleavage with hydrogen transfer, and other fragment ions which result from cleavage of the ribose.[13] The requirement of thermal evaporation prior to ionization makes chemical ionization, as well as electron impact, an unsuitable method for analyzing thermally labile nucleosides, such as 7-methylguanosine. Mass spectra of these nucleosides may be obtained with the aid of derivatization such as permethylation,[14] trimethylsilylation,[15] and acetylation,[16] which enhances volatility and increases thermal stability.

An alternative to derivatization is the use of ionization methods which sample directly from the solid phase. Among these, field desorption[17] and plasma desorption[18] have seen some application to nucleotides and related compounds. The latter technique has achieved striking successes but requires highly specialized instrumentation so interest in simpler alternatives is justified. In secondary-ion mass spectrometry, the method used in this study, the solid sample is bombared by an energetic particle beam (typically argon ions) and the ejected secondary ions are mass analyzed. The technique has emerged as a very sensitive procedure which yields characteristic mass spectra, including molecular ions, for thermally labile biological compounds.[5,6,19] Spctra are stable for long periods of time, sometimes hours, and sample preparation is simple, both features which establish contrasts between SIMS and field desorption. Sensitivity is maximized when the analyte exists as a preformed salt rather than as a neutral molecule since the latter must be both desorbed from the surface and ionized, by cation attachment for example. This appears[20] to be a feature common to all the desorption/ionization techniques and it is utilized herein to increase ion yields by examination of an appropriate form of the analyte.

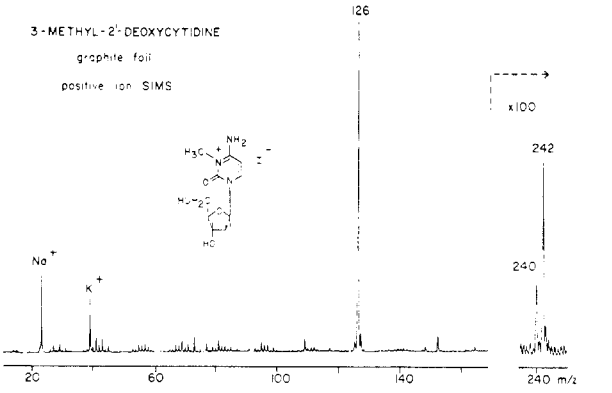
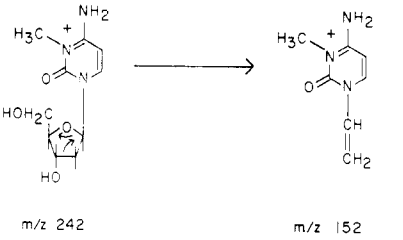


Figure 1. SIMS spectrum of title deoxyribonucleoside in salt form, showing intact cation (242^+), dehydrogenation product (240^+), and the intact base (126^+).

Scheme I



Results and Discussion

The SIMS spectrum of 3-methylcytidine on a platinum foil is presented in the accompanying paper.[7] The spectrum is exceedingly simple, exhibiting as the only intense ions the intact methylated nucleoside at m/z 258 and the 3-methylcytosine ion at m/z 126 generated by glycosidic cleavage with hydrogen transfer to the base. This fragment ion, bH_2^+ in McCloskey's nomenclature,[12] characterizes the base and is a focus of interest in this study. Deoxynucleosides may also be characterized by SIMS; the spectrum of 3-methyl-2′-deoxycytidine on graphite (Figure 1) yields ions analogous to those observed for the ribonucleoside. Both the intact modified deoxyribonucleoside at m/z 242 and the 3-methylcytosine ion at m/z 126 are present with abundances similar to those observed in the SIMS spectrum of the ribonucleoside. Loss of H_2 from the intact molecule accounts for the 240^+ ion. This process is often observed in the SIMS spectra of various compounds, including sugars,[20] and it is also observed for the other modified nucleosides discussed herein. It is difficult, however, to distinguish H_2 loss from a possible oxidation of the ribose sugar in the the course of ion impact on the sample. The increase in intensity of 240^+ observed with primary ion flux and the time necessary to record the spectrum suggest the latter process may contribute.

The quality of the SIMS spectra of nucleosides is such that even minor peaks have assignable origins. For ex-

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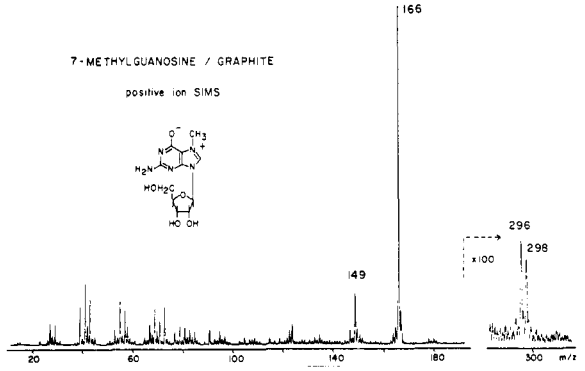


Figure 2. SIMS spectrum of the title nucleoside, showing the intact nucleoside and its dehdyrogenation product (298^+ and 296^+, respectively as well as the base characteristic ion 166^+ and fragment (149^+).

ample, loss of 17 amu from the 3-methyl-2′-deoxycytosine ion occurs to yield m/z 109 with approximately 5% of the intensity of the modified base peak (Figure 1). This process may be ascribed in part to OH· elimination based on the pattern of its occurrence in the various modified bases and on the analogy with CH_3· loss also seen in some systems.

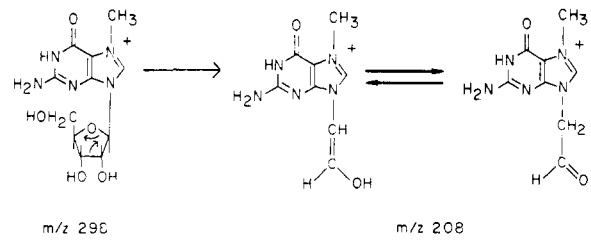
Two other noteworthy fragment ions observed at low intensity in the spectrum of 3-methyl-2′-deoxycytidine occur at m/z 148 and 152 (not labeled in Figure 1). They correspond to the sodium-cationized base (attachment of Na^+ instead of H^+) and to the base plus a C_2H_3 deoxyribose fragment, respectively. The latter process (Scheme I) mimics that observed in electron-impact mass spectrometry.[12] Both these ions occur in other compounds and may be used to confirm the molecular weight of the modified base. Sodium cationization of the base does not occur when the concentration of Na^+ is low as evidenced in the SIMS spectrum of 3-methylcytidine (in the accompanying paper); however, as expected and shown below, by deliberate addition of a cationizing ion such as Na^+ or K^+, multiple checks on the molecular weight of the alkylated base may be made.

Figure 2 presents the SIMS spectrum of 7-methylguanosine, a compound which is particularly unstable thermally. The spectrum is analogous to those already discussed, providing both molecular weight and structural information in the form of the intact 7-methylguanosine ion at m/z 298, along with three major fragment ions. Loss of H_2 from the intact cation accounts for 296^+, while glycosidic cleavage with hydrogen transfer generates the 7-methylguanine ion at m/z 166. Loss of 17 amu from 166^+ is assumed to produce the ion at m/z 149, observed with approximately 10% intensity relative to the base peak. An ion (m/z 188) corresponding to the attachment of sodium to the zwitterionic form of the modified 7-methylguanine base is present in some spectra. The relative intensity of the sodium-cationized base to other ions observed in the SIMS spectrum varies depending upon the concentration of Na^+ in the sample. Indeed, addition of NaCl to the sample matrix dramatically increases $(B + Na)^+$. In this ribonucleoside the base plus $C_2H_3^+$ ion occurs to a minor extent compared to C_2H_3O attachment (m/z 208, relative abundance 2–5%). This appears to characterize the sugar and the generation of m/z 208 can be postulated to arise by a process (Scheme II) which parallels that occuring in electron impact and in chemical ionization.[12,21]

Recently, plasma-desorption mass spectrometry has been used to acquire a spectrum of 7-methylguanosine.[22]

(21) Wilson, M. S.; McCloskey, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 3436.

Scheme II



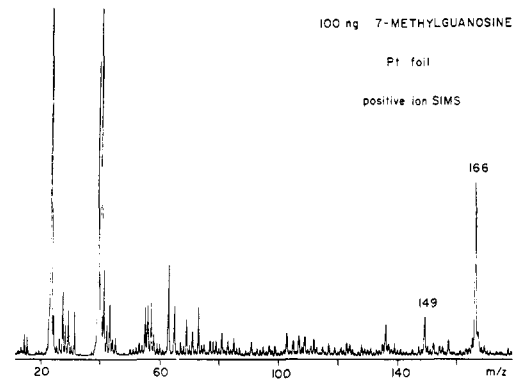


Figure 3. A portion of the SIMS spectrum of 100 ng of 7-methylguanosine on a 1-cm^2 metal support, illustrating signal-to-noise characteristics.

Table I compares the SIMS spectrum of 7-methylguanosine with that obtained by plasma desorption, including the relatively minor ions due to base attachment processes discussed above. The resemblance is striking. Not only are identical ions observed, but similar relative ion intensities are seen. Considering the vary different phenomena upon which plasma desorption[23] and SIMS[5,24,25] are based, the observation of nearly identical spectra for 7-methylguanosine helps to confirm the close mechanistic relationship suspected to exist between the two ionization methods. Other comparisons of this type lead to the same conclusion.[26,27] A spectrum of 7-methylguanosine has also been obtained by field-desorption mass spectrometry[28] and shows both the 7-methylguanine ion at m/z 166 (100%) and the protonated molecule at m/z 298 (11%). No molecular ion, M^+·, was observed, but an ion corresponding to $(M - 17)^+$ at m/z 280 was observed at 30% relative intensity.

Since SIMS is a surface-sensitive technique, probing only the uppermost 10–20 Å, analysis conditions are not precisely reproducible and quantitation must be performed with an internal standard. This is best done by using an isotopically labeled form of the analyte, and use of this procedure for 3-methylcytidine is described in the accompanying paper.[7] Other closely related compounds can also serve as internal standards. For example, 7-methylguanosine (400 ng) was quantified by ion bombardment of a mixture with [*methyl*-$^2H_3$]-7-methyl-2′-deoxyguanosine (50 ng). The observed abundance ratio of modified base peaks, $166^+/169^+$ was 7.5 ± 0.5, and the

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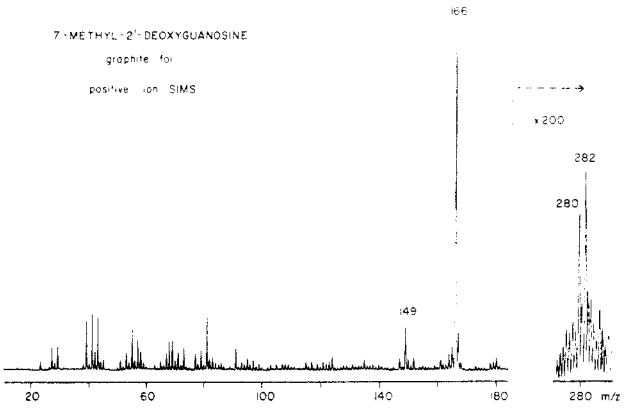

Figure 4. Spectrum of 7-methyl-2′-deoxyguanosine, illustrating similarity to behavior of 7-methylguanosine.

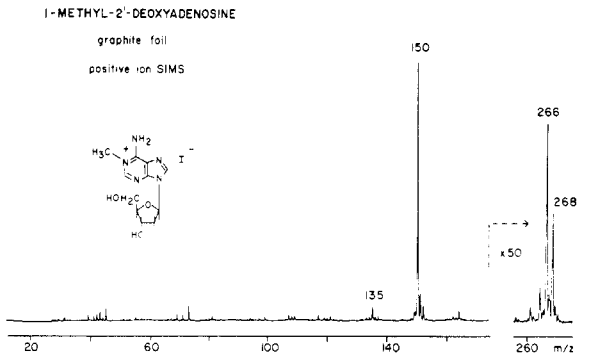

Figure 5. Title compound shows base characteristic ions (150^+, 135^+), hydrogenated nucleoside (268^+), and the intact nucleoside (266^+).

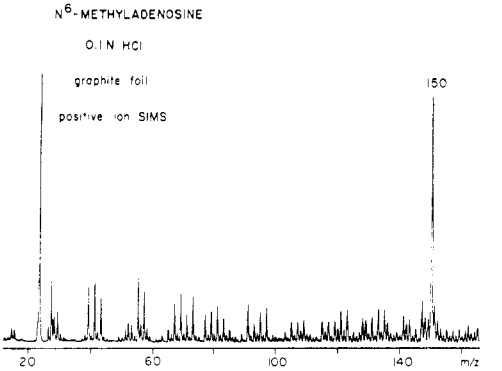

Figure 6. Acid treatment gives spectrum of N^6-methyladenosine which includes the ion m/z 150, which characterizes the base.

expected mole ratio is 7.7. The result encourages further experiments in both detection and quantitation of 7-methylguanosine and other nucleosides at nanogram levels by using SIMS.

For illustration of the spectral quality which may be obtained with nanogram quantities of a modified nucleoside, Figure 3 shows part of the SIMS spectrum of 100 ng of 7-methylguanosine. With the exclusion of Na^+ and K^+ ions (mostly background), this spectrum is of comparable quality to that obtained with 10–20 μg of the analyte. Signal intensity, however, decreases rapidly as the amount of analyte is further decreased. For 7-methylguanosine, 10 ng produced a signal-to-noise ratio of approximately 4:1, while 1 ng of the analyte was not detectable.

The deoxyribonucleoside, 7-methyl-2′-deoxyguanosine, showed (Figure 4) similar behavior to the ribonucleoside upon argon ion bombardment. A secondary ion corresponding to the intact modified nucleoside is seen at m/z 282, and the ubiquitous H_2 loss accounts for 280^+. These ions are present at low signal to noise ratios (approximately 4:1) and at 0.3% intensity relative to the base peak (166^+). The latter, protonated 7-methylguanine (m/z 166), is formed by glycosidic cleavage with hydrogen transfer, while hydroxyl or ammonia loss account for 149^+. It is noteworthy that the relative ion intensities in the deoxyribonucleoside spectrum are similar to those in the ribonucleoside.

Both Na^+ and $C_2H_3^+$ addition forms ions observed (Figure 4) at approximately 1–2% intensity relative to the 7-methylguanine ion at m/z 166. No $C_2H_3O^+$ adduct is seen for the deoxynucleoside, confirming that this ion results from fragmentation of the ribose sugar as illustrated in Scheme II.

The SIMS spectrum of another modified nucleoside, 1-methyl-2′-deoxyadenosine is shown in Figure 5. The presence of 1-methyl-2′-deoxyadenosine as the iodide salt enhances its sensitivity for analysis by SIMS. The base fragment of 1-methyl-2′-deoxyadenosine, the 1-methyladenine ion, is observed at m/z 150, while methyl radical loss from 150^+ accounts for 135^+ (compare the loss of hydroxyl radical from 7-methylguanosine). An ion at m/z 266 of approximately 2% intensity relative to 150^+ is attributed to the intact methylated nucleoside, while some H_2 loss is observed at m/z 264. The ion at m/z 268 apparently represents a reduced form of the nucleoside. This proposal is supported by the relatively large (approximately 6% of 150^+) ion at m/z 152, which is assigned as the reduced form of the modified base. Analogous results have been reported for chemical ionization by McCloskey and co-workers.[21] No other alkylated nucleosides studied herein exhibit ions corresponding to reduced forms of the base of nucleoside. No sodium-cationized adduct is observed with this base, yet the $C_2H_3^+$ adduct is present at m/z 176 at equal intensity to 266^+.

Methylation of the N^6-amino instead of the N^1 position of adenosine yields a neutral compound rather than a salt. As such, the sensitivity for analysis by SIMS is dramatically reduced. However, addition of 0.1 N HCl to the analyte matrix to preform the acid salt improves the spectral quality, albeit at the expense of cleaving the base–ribose bond. Figure 6 shows the SIMS spectrum of N^6-methyladenosine in the presence of 0.1 N HCl on graphite. The protonated modified base is observed at m/z 150, while no other fragment ions may be distinguished from the background.

A similar result is obtained for the corresponding mononucleotide. Figure 7 shows the SIMS spectrum of N^6-methyladenosine 5′-monophosphate neat (top) with that generated by the addition of acid to the analyte (bottom). In this compound, disodium phosphite and disodium phosphate 125^+ and 143^+, are produced under ion bombardment and obsure the analyte contribution to the mass spectrum. Addition of 0.1 N HCl to the sample matrix enhances 150^+ by a factor of 5 by preforming acid salt. Acid enhancement of SIMS spectra must, of course, be used with caution when isomer distinction is required.

Methylation of the ribose sugar results in the production of $O^{2'}$-methyladenosine whose SIMS spectrum is distinguished from both the 1-methyl or N^6-methyl isomers by the presence of 135^+ as the base peak. Neither the 1-methyl or the N^6-methyl isomers show 135^+ as a dominant ion. For the $O^{2'}$-methyl isomer no intact methylated nucleoside was seen, in contrast to the behavior of most other systems studied.

Extensions to Polynucleotides. The techniques described here, including both SIMS and the other desorption/ionization procedures, should be applicable to the

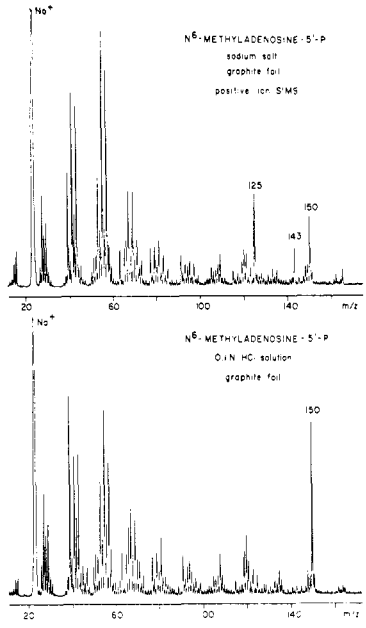

Figure 7. Effect on spectral quality when the nucleotide is chemically modified by acid pretreatment.

characterization of polynucleotides including DNA and RNA. Two approaches can be distinguished, one involving enzymatic degradation to mononucleotides or mononucleosides followed by mass spectrometry and the other being direct pyrolysis of the biopolymer. If this latter approach is followed by a technique such as mass spectrometry/mass spectrometry, capable of handling complex mixtures, then the procedure is attractive because of its extreme simplicity. Pyrolysis MS/MS has indeed been used to generate spectra of modified bases from DNA;[9,10] however, ambiguity regarding methyl rearrangements has generated questions[29,30] about this procedure. For many nucleosides the question of methyl rearrangements is of course critical since the alkylation site may be as important as the identification of the presence of the modified base.

We have repeated the experiments and confirm earlier claims[10] that mass spectrometry/mass spectrometry used with heated direct probe sample introduction and chemical ionization serves to distinguish 1-methyladenine from 2-methyl and N^6-methyl isomers. In addition, it has been confirmed that pyrolysis of intact salmon sperm DNA gives a spectrum which matches that of 1-methyladenine. In light of subsequent work by McCloskey and co-workers[29] we no longer conclude that this result requires the presence of 1-methyladenine base units in the DNA. Indeed, we find that pyrolysis of poly(dAdT)·poly(dAdT) alternating copolymer procedures detectable quantities of 1-methyladenosine which persist at probe temperatures above 300 °C. Although the time and temperature profiles for 1-methyladenine production differ between the DNA and synthetic copolymer samples, the explanation that methyl rearrangement occurs during pyrolysis reasonably accounts for the modified base detected from DNA.

Conclusion

Secondary-ion mass spectrometry, used with the simple preparation procedure described here, provides mass spectra from which the mass of the nucleoside and its constituent base can be identified. Fragment ions, except for the base ion itself, are of low abundance, but they show mechanistic similarities to processes observed in electron impact and chemical ionization. This suggests that isomer distinction should be feasible. Priliminary results have been obtained for laser-desorption MS/MS which support this as well as demonstrating similar spectra to those obtained by SIMS. Sensitivity and quantitative accuracy of the SIMS technique are good and encourage application to analysis of polynucleotide methylation products after enzymatic depolymerization. This approach has been used in quantitating the degree of methylation of a synthetic homopolymer (accompanying paper).

Changes in the matrix from which SIMS is performed is known to have pronounced and sometimes favorable effects on the spectra;[20] future efforts will be directed at increasing the intensity of the nucleoside ions relative to the base-characteristic ions by using this approach.

Experimental Section

The synthesis of 3-methylcytidine is presented in the accompanying paper.[7] Preparation of 3-methyl-2′-deoxycytidine (m^3dC) was accomplished match that given (0.495 g, 2.18 mM) in 3 mL of dry Me_2SO and adding methyl iodide (2.180 g, 15.35 mM) in Me_2SO solution (1 mL). The reaction proceeded at room temperature and the mixture was stirred for 6 h; the reaction was monitored by using silica thin-layer chromatography plates eluted with an 2-propanol/ammonia/water (6:3:1) mixture (R_f valves, dC = 0.69, m^3dC = 0.78). The reaction mixture was added to 100 mL of cold acetone, and the suspension was stored at 4 °C overnight. The precipitate was filtered and dryed in vacuo to yield 0.724 g (90%) of dry 3-methyl-2′-deoxycytidine: ^{13}C NMR (Me_2SO-d_6) δ 159.2 (C_4), 147.8 (C_2), 141.8 (C_6), 94.4 (C_5), 30.9 (3-methyl); ^1H NMR (Me_2SO-d_6) υ, 8.2 (d, J_{6-5} = 7.9 Hz, H_6, 6.2 (d, J_{5-6} = 7.9 Hz, H_5, 6.2 (t, J = 5.6 Hz $H_{1'}$ 3.4 (*N*-methyl); mp 145 °C (iodide salt); UV (H_2O) max 275 nm, min 238 nm.

Other alkylated nucleosides including 1-methyl-2′-deoxyadenosine, 7-methyl-adenosine, and [*methyl*-$^2H_3$]-7-methyl-2′-deoxyguanosine have been synthesized according to ref[32]. Melting point and UV absorption data match that given in the literature. Sigma Chemical Co. supplied both $O^{2'}$-methyladenosine and 7-methylguanosine, while N^6-methyladenosine monophosphate and 1-methyladenosine monophosphate were obtained from Aldrich Chemical Co.

Spectra were recorded with a Riber SIMS system which has been previously described.[5] The analyte was deposited from aqueous solution onto a platinum or graphite foil so that approximately 10 μg of the modified nucleoside was present. The solvent was subsequently evaporated and the sample was introduced into the vacuum chamber for analysis.

Ion bombardment (Ar^+, 5 keV; 1×10^{-9} A/cm^2) of the absorbed analyte produces secondary ions, which are analyzed with a Riber SQ156 quadrupole mass filter. The ions are detected by using a Galileo 4830 Channeltron electron multiplier and the resulting signal is processed with a Princeton Applied Research 1121 amplifier/discriminator. The spectra are directly recorded on a Hewlett-packard Model 7041A chart recorder and are not corrected for background.

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