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This Research Contribution is in Commemoration of the Life and Science of I. M. Kolthoff (1894–1993).

Gas-Phase Ion–Molecule Reactions: A Model for the Determination of Biologically Reactive Electrophilic Contaminants in the Environment

Jody A. Freeman,† Jodie V. Johnson,* and Richard A. Yost
Chemistry Department, University of Florida, Gainesville, Florida 32611

Douglas W. Kuehl
U.S. Environmental Protection Agency, Duluth, Minnesota 55804

A promising instrumental technique has been investigated to rapidly screen complex environmental samples for chemical contaminants having the propensity to covalently bond to biomacromolecules such as DNA. Radical molecular ions of pyridine, a model compound for nucleophilic bases of DNA, were mass-selected and allowed to react with electrophilic environmental contaminants in the collision cell of a triple quadrupole mass spectrometer. Analytes were introduced into the collision cell via a gas chromatographic column. Reactive chemicals are then characterized by scanning Q3 to identify associative reaction products. A good qualitative correlation was observed for the gas-phase reactivity of a series of electrophilic reagents with both their alkylating reactivity in solution (4-(4-nitrobenzyl)pyridine) and AMES test mutagenicity which had been previously published. Femtomole limits of detection for specific associative reaction products were demonstrated. Gas-phase reactions of ions of environmental contaminants (introduced into the source) with neutral pyridine (in the collision cell) were also investigated. Reactions of the radical molecular ion of the allyl reagents with neutral pyridine were similar to results from the mass-selected reaction of the pyridine radical molecular ion with neutral allylic reagents.

An ever-increasing demand exists to develop highly sensitive and rapid analytical techniques for the characterization of chemical contaminants in environmental samples. For the most part, this need has been fulfilled during the past few years with the development of the state-of-the-art computerized gas chromatography/mass spectrometry (GC/MS) systems. These systems contain large libraries of mass spectra that may be rapidly and efficiently searched to identify and quantify chemicals. More recently, however, the emphasis of environmental monitoring has focused on chemical contaminants that are biologically active,[1] including those chemicals that have the potential to be mutagenic or carcinogenic. This need presents a unique challenge of selectively characterizing only those chemicals in a complex mixture that have the propensity to react with key biomacromolecules, such as DNA. Perhaps the best-known method to screen for reactive chemicals is the Ames test.[2–4] This test has been shown to be an effective monitor for mutagenic potential; however, analyte-specific information cannot be obtained from complex mixtures unless contaminants are first isolated and then individually tested. This requirement necessarily limits information to a whole sample or minimally to multicomponent fractions of the sample. If chemical specific data is required, each contaminant must either be carefully identified, and estimates of toxic potential made, or be isolated for biological testing. Each of these tasks is very difficult and therefore time-consuming and expensive. We have instead chosen to use, in a unique application, the sensitivity, selectivity, and potential for rapid and low cost analysis of gas chromatography/tandem mass spectrometry (GC/MS/MS) to solve this problem. Our approach is to use mass-selective ion–molecule reactions in the center collision chamber of a triple quadrupole mass spectrometer to screen complex environmental samples for potentially reactive compounds. This paper describes more fully the development and evaluation of ion–molecule reactions we have preliminarily reported for these analyses.[5]

We have chosen to initiate these studies using a series of directly reactive chemicals, i.e., those chemicals that do not require metabolic activation to covalently bond to DNA: allyl isocyanate, allyl chloride, allyl bromide, and allyl iodide. Although these chemicals are not expected to persist in the environment, they are an excellent set of electrophilic (electron-deficient) chemicals with which to begin our studies because aqueous solution-phase reactivity and Ames test data already exist with which to compare the gas-phase ion–molecule reactivity data.[6] Pyridine was chosen to represent a nucleophilic (electron-rich) biomacromolecule, such as DNA.

Analogous, but not similar, approaches to the use of gas-phase ion–molecule reactions have been reported in the literature. For instance, Davoli and co-workers have studied gas-phase reactions of pyridine and guanine with polynuclear

† Current address: U.S. Environmental Protection Agency, Cincinnati, OH 45268.

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Table 1. Gas Chromatography Conditions Employed in Ionized Nucleophile/Neutral Electrophile and Ionized Electrophile/Neutral Nucleophile Reactions

	GC column	temperature program	split ratio
	$N^{\bullet+}$/E Reactions		
reactivity of $N^{\bullet+}$ ions	J&W 6 m × 0.178 mm i.d. × 0.4 μm film thickness	25 °C to 150 °C at 20 °C/min after 1 min	30:1
calibration studies	J&W 20 m × 0.178 mm i.d. × 0.4 μm film thickness	50 °C to 100 °C at 20 °C/min	100:1
gas-phase reactivity	J&W 20 m × 0.178 mm i.d. × 0.4 μm film thickness	50 °C to 200 °C at 10 °C/min after 1 min	100:1
	$E^{\bullet+}$/N Reactions		
reactivity of $E^{\bullet+}$ ions	Perkin Elmer 15 m × 0.32 mm i.d. × 1.0 μm film thickness	30 °C to 200 °C at 15 °C/min	20:1
calibration studies	Perkin Elmer 15 m × 0.32 mm i.d. × 1.0 μm film thickness	25 °C to 70 °C at 20 °C/min	20:1
gas-phase reactivity	J&W 20 m × 0.178 mm i.d. × 0.4 μm film thickness	25 °C to 200 °C at 10 °C/min	100:1

aromatic hydrocarbons (PAHs) in the ion source of a tandem mass spectrometer.[7] Whitehill, Mathai, and Gross have recently begun research to determine the correlation of gas-phase reactivity of radical cations of aromatic hydrocarbons and nitrogen-containing models for DNA bases such as imidazole.[8] In addition, gas-phase synthesis of methylated nucleosides in the ion source of a mass spectrometer has been reported.[9] In the last 2 decades, various attempts have been made to relate biological activity to molecular structure with quantitative structure–activity relationships (QSARs); these techniques have been reviewed in the recent literature.[10–12] QSARs combined with GC/MS for rapid characterization of chemical contaminants in environmental samples are used by regulatory agencies and industry to make rapid and cost-effective predictions about the toxicity and reactivity of industrial chemicals. The U.S. Environmental Protection Agency is using QSAR-GC/MS data in the premarket notification process for the registration of chemicals and for the assessment of chemicals at contaminated sites such as hazardous waste sites. The disadvantages of QSAR-GC/MS are as follows: first, each contaminant must be correctly identified before predictions can be made, and second, tremendous volumes of data can be rapidly produced by a GC/MS system, which can quickly overwhelm data reviewers.

This paper presents an evaluation of the analytical utility of mass-selected ion–molecule reactions between pyridine and a series of electrophilic alkylating compounds in the collision cell of a triple quadrupole mass spectrometer and describes a method to measure reactivity/amount of contaminant. These reactions provide a rapid and sensitive technique for both screening complex environmental samples and estimating the propensity for mutagenic or carcinogenic adverse effects.

EXPERIMENTAL SECTION

Instrumentation. Initial experiments were performed on a Finnigan MAT TSQ 70 triple quadrupole mass spectrometer equipped with a Varian (Walnut Creek, CA) Model 3400 gas chromatograph. The TSQ 70 was later upgraded with an octopole collision cell and a high-voltage (20 kV) conversion dynode. These changes did not affect the results. Gas chromatography was carried out on a J&W Scientific (Folsom, CA) DB-5 or a Perkin Elmer (Norwalk, CT) bonded methyl silicone capillary column in the split mode with helium carrier gas at an inlet pressure of 5 psig and with injection port and transfer line temperatures of 200 °C. GC parameters are listed in Table 1. All experiments were performed at an emission current of 200 μA, electron energies of 70 eV (electron ionization, EI) or 100 eV (chemical ionization, CI), a manifold temperature of 100 °C, and ion source temperatures of 170 °C for EI or 150 °C for CI.

A GC/MS transfer line designed by Hail and co-workers[13] was modified so that it was resistively heated up to the vacuum chamber feed-through while the portion inside the vacuum system was heated only by the vacuum manifold heater (100 °C).

The mass spectrometer was mass-calibrated using perfluorotributylamine (FC43) and optimized for maximum ion–molecule reaction product yield in the collision cell. A constant flow of either allyl chloride (for ionized nucleophile/neutral electrophile studies) or pyridine (for ionized electrophile/neutral pyridine studies) was introduced via the collision gas inlet into the collision cell using either a Negretti fine-metering valve (Hampshire, England) or a variable leak valve (Granville Phillips, Model 203). Collision energy, collision cell rf potential, and the potentials on the lenses immediately before and after the collision cell were optimized for maximum transmission of the Q1 mass-selected ion and the product ion (e.g., 120^+). Typical collision energies were 2 or 3 eV.

Samples and Reagents. Propyl bromide, propyl chloride, and benzyl chloride were purchased from Eastman Kodak (Rochester, NY). Allyl chloride, allyl iodide, allyl isothiocyanate, allyl bromide, and 2,3-dichloropropene were purchased from Aldrich Chemical Co. (Milwaukee, WI), and pyridine was purchased from Fisher Scientific (Fair Lawn, NJ). The solvents, HPLC-grade pentane and Spectro-grade heptane, were purchased from Fisher Scientific and Eastman Kodak, respectively. From analytical standards, the following were prepared: (1) an equal molar (30 nmol/μL) each of allyl chloride, allyl bromide, allyl isothiocyanate, benzyl chloride, 2,3-dichloropropene, propyl bromide, and propyl chloride in pentane; (2) allyl chloride and allyl iodide at concentrations of 1, 2.5, 5, 10, 25, 50, 75, 100, and 500 ng/μL and 5, 10, and 30 μg/μL in pentane; (3) allyl chloride, allyl bromide, and allyl isothiocyanate (6 μg/μL) in heptane; and 4: allyl iodide (6 μg/μL) in pentane.

Mass-Selected Ionized Nucleophile/Neutral Electrophile Reactions in Collision Cell. This section describes reactions

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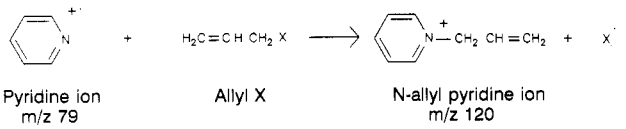

Figure 1. Collision cell reactions of the mass-selected $N^{\cdot+}$ of pyridine with neutral allyl compounds of the general form C_3H_5X, where X is NCS, Cl, Br, or I.

in the collision cell between mass-selected ions of the model nucleophile (N), pyridine, and neutral electrophiles (E) (Figure 1). Pyridine was introduced into the ion source through a Granville Phillips (Boulder, CO) variable-leak valve attached to a hollow stainless steel probe shaft (1/2-in. o.d.) inserted into the vacuum system through the conventional solids probe lock and ionized under EI or methane CI conditions. The pyridine reactant ion was mass-selected by the first quadrupole (Q1) and allowed to react in the collision cell with neutral electrophilic reagents eluting from the GC column, and product ions were mass-analyzed by scanning the third quadrupole (Q3).

Relative Reactivity of Pyridine Ions. Relative reactivity of various pyridine ions with neutral electrophiles in the collision cell was investigated. EI conditions (EI ion volume) were used to generate the molecular ions of pyridine ($N^{\cdot+}$) and methane ($CH_4^{\cdot+}$). Positive and negative CI conditions (CI ion volume, 1.6 Torr CH_4) were used to generate the $[N + H]^+$, $[N + C_2H_5]^+$, $[N + C_3H_5]^+$ or $[N - H]^-$ ions of pyridine and the CH_5^+ ion of methane. Pyridine and methane ions were alternately mass-selected by Q1. Mass spectra were obtained at an EM voltage of −1000 V, and Q3 was scanned from 30 to 300 amu in 0.25 s. Analyzer high vacuum pressure was 1.2×10^{-5} Torr. Test mixtures 3 and 4 were analyzed in triplicate.

Calibration Studies. Pyridine was introduced into the ion source at a constant pressure of 2×10^{-4} Torr (as measured by a Bayard–Alpert ionization gauge mounted on the ion source vacuum manifold) and ionized under EI conditions. Selected reaction monitoring (SRM) of the product ion was performed by scanning Q3 from 119.5 to 120.5 amu in 0.25 s. Product ions were detected at an EM voltage of −1600 V. Test mixture 2 was analyzed in triplicate.

Relative Reactivity of Neutral Electrophiles. Pyridine $N^{\cdot+}$ ions and methane $CH_4^{\cdot+}$ ions (1.2 Torr, EI ion volume) were alternately mass-selected to react with neutral electrophilic reagents. Q3 was scanned from 30 to 300 amu in 0.25 s, and product ions were detected at an EM voltage of −1200 V. The pyridine ion source pressure without methane present was 2.0×10^{-4} Torr as measured by the ionization gauge mounted on the ion source vacuum manifold. Test mixture 1 was analyzed in triplicate.

Mass-Selected Ionized Electrophile/Neutral Nucleophile Reactions in Collision Cell. This section describes reactions in the collision cell between electrophilic reagent ions and neutral pyridine. In these experiments, the electrophilic reagents were introduced into the ion source via a GC column and ionized by either EI or CI. Pyridine was present in the collision cell at 1.0–1.5 mTorr (high vacuum of 8×10^{-6} Torr) as measured by a Granville Phillips convectron gauge. As exemplified by the two reactions given in Figure 2, an electrophile ion was mass-selected by Q1 for reaction with

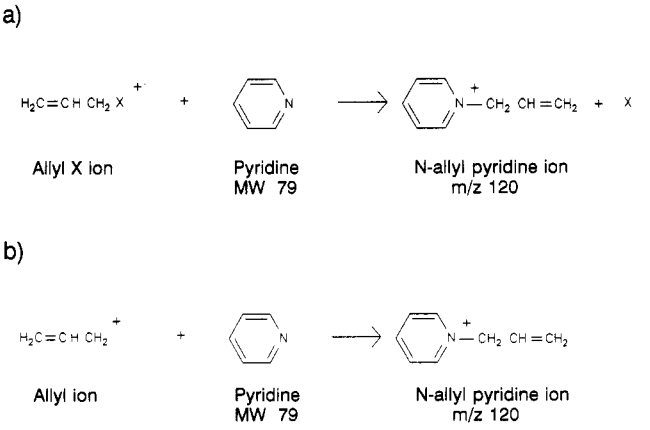

Figure 2. Reactions of (a) the $E^{\cdot+}$ ion and (b) the $[E - X]^+$ ion of allylic compounds of the general form C_3H_5X, where X is NCS, Cl, Br, or I, with neutral pyridine in the collision cell.

pyridine in the collision cell, and the product ions were mass-analyzed with Q3. The mass-selected reactions of the EI-generated radical molecular ion ($E^{\cdot+}$) of each electrophilic reagent and the allylic ion ($C_3H_5^+$, $[E - X]^+$) produced from the EI fragmentation of the allyl reagents and the methane CI-generated $[E + H]^+$, $[E + C_2H_5]^+$, and $[E + C_3H_5]^+$ ions of each electrophilic reagent were studied.

Relative Reactivity of Ionized Electrophilic Reagents. The relative reactivity of the EI- and CI-generated ions from each electrophilic reagent with neutral pyridine was investigated. The ion intensities of the electrophile ions before the reaction and of the product ions resulting from the reaction with pyridine were obtained by alternating Q1 scans and mass-selected reaction Q3 scans, respectively. The ions of each electrophile were mass-selected for reaction with pyridine only during that electrophile's retention time window. Q1 mass spectra were obtained from m/z 35 to 280 in 0.15 s with an EM voltage of −800 V. Q3 mass spectra were obtained from m/z 30 to 300 in 0.25 s with an EM voltage of −1000 V. Triplicate injections of test mixture 4 were analyzed.

Calibration Studies. The radical molecular ion ($E^{\cdot+}$) of each allyl reagent was produced (EI) and mass-selected by Q1, and the reaction product (pyridine + C_3H_5)$^+$ was analyzed by scanning Q3 from m/z 119.5 to 120.5 amu in 0.25 s in the selected reaction monitoring mode (SRM) and from m/z 30 to 280 in 0.25 s for full-scan detection limits. Product ions were detected at an EM voltage of −1600 V. Test mixture 2 was analyzed in triplicate.

Gas-Phase Reactivity Studies. The allyl reagents were ionized in the ion source under EI conditions. Data were obtained by alternating between a Q1MS scan and mass-selection of the $E^{\cdot+}$ ions of the eluting compounds. For the Q1MS scan, Q1 was scanned from m/z 30 to 300 in 0.20 s at an EM voltage of −800 V. During Q1 mass-selection of the $E^{\cdot+}$ ion, Q3 was scanned from m/z 30 to 300 in 0.20 s at an EM voltage of −1400 V. Test mixture 1 was analyzed in triplicate for each experiment.

RESULTS AND DISCUSSION

Mass-Selected Ionized Nucleophile/Neutral Electrophile Reactions in Collision Cell. Relative Reactivity of Pyridine Ions. The reactivity of the ionized nucleophile (N) produced

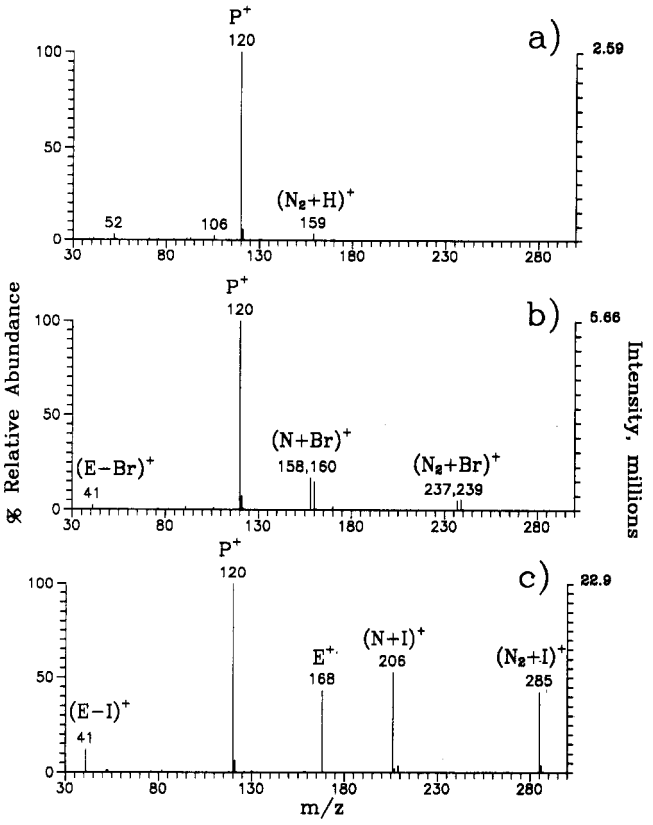

Figure 3. Background-subtracted mass spectra obtained from the reaction of the $N^{\cdot+}$ ion of pyridine with (a) allyl chloride, (b) allyl bromide, and (c) allyl iodide.

in the ion source under EI and CI conditions is of both fundamental and practical interest. Pyridine ions, $N^{\cdot+}$, $[N + H]^+$, $[N + C_2H_5]^+$, $[N + C_3H_5]^+$, and $[N - H]^-$, were allowed to react with allyl isothiocyanate, allyl chloride, allyl bromide, and allyl iodide. However, only $N^{\cdot+}$ reacted with neutral analytes to produce the associative reaction product ions of interest. The fact that the other positive ions did not react suggests that the site of reactivity on pyridine was blocked by the attachment of a proton or an alkyl group.

Background-subtracted mass spectra of the products of the reaction of $N^{\cdot+}$ with allyl chloride, allyl bromide, and allyl iodide are shown in Figure 3. The product ion, m/z 120, was the base peak in each mass spectrum. Because the mass spectra of Figure 3 have been background subtracted, the unreacted mass-selected 79^+ ion of pyridine, which would have been the base peak, has been subtracted out of the mass spectra. Comparison of the base peak intensities indicated that the order of reactivity was allyl chloride < allyl bromide < allyl iodide. Since the formation of the product ion involved the breaking of a carbon–halide bond, the internal energy remaining in the resulting product ion would depend upon the energy required to break this bond. The carbon–halide bond strength decreases in the order Cl > Br > I. Thus, the relative internal energy remaining in the product ion formed by reaction with allyl halides would be in the reverse order, i.e., the product ions formed by reaction of $N^{\cdot+}$ with allyl iodide would have more internal energy and be less stable than the product ion resulting from the reaction of $N^{\cdot+}$ with allyl bromide, which would be less stable than the allyl chloride product ion. This also resulted in the increasing intensity of the $C_3H_5^+$ ion (m/z 41) in going from the allyl chloride to the allyl bromide to the allyl iodide mass spectra. The allyl bromide mass spectrum contained $[N + {}^{79}Br]^+$ and $[N + {}^{81}Br]^+$ ions (m/z 158 and 160, respectively), which undergo further reaction with neutral pyridine present in the collision cell to form the $[N_2 + Br]^+$ ions at m/z 237 and 239, respectively. Allyl iodide was observed to undergo analogous reactions to give m/z 206 and 285, while allyl chloride did not. Eberlin and co-workers reported similar halogen-bound pyridine products.[14] Due to their similar ionization energies (IE), charge exchange can occur between neutral allyl iodide (IE = 9.298 eV) and the pyridine $N^{\cdot+}$ ion (IE = 9.25 eV) to produce the radical molecular ion of allyl iodide ($E^{\cdot+}$) at m/z 168. Allyl chloride (IE = 9.9 eV) and allyl bromide (IE = 10.06 eV) do not undergo charge exchange with pyridine since their ionization energies are above that of pyridine.

Analyte Identification and Quantitation. With the use of the $N^{\cdot+}$ reaction scans to screen complex mixtures for the presence of genotoxic compounds, a method is also required to identify and quantitate each component so that their relative reactivity can be established. With mass-selected reactions, a reactant ion can be chosen that is more universal in its ionizing capabilities than pyridine $N^{\cdot+}$. In these experiments, $CH_4^{\cdot+}$ and $N^{\cdot+}$ were mass-selected for reaction with neutral analytes in the collision cell during alternating scans. Background-subtracted product mass spectra obtained by reaction with $CH_4^{\cdot+}$ with allyl chloride, allyl bromide, and allyl iodide are shown in Figure 4. Although charge exchange with these allylic compounds produces $E^{\cdot+}$ and $[E - X]^+$ ions (X = Cl, Br, I), product spectra are dominated by pyridine ions ($[N + H]^+$, $[N_2 + H]^+$) and pyridine–allyl halide product ions (P^+). Due to the relatively high pressure of pyridine in the source, some neutral pyridine was also present in the collision cell. Ionization of this pyridine by $CH_4^{\cdot+}$ charge exchange and the formation of unwanted pyridine/electrophile product ions make the identification of the analyte difficult. By reducing the pyridine pressure in the source (and therefore in the collision cell), the amount of these unwanted reactions was decreased; this also reduced the amount of the $N^{\cdot+}$ reaction products formed during the $N^{\cdot+}$ scans. However, since all the ions present in the background-subtracted $CH_4^{\cdot+}$ product spectra are a function of the electrophile, mass chromatograms of these ions can be used to normalize the $N^{\cdot+}$/E gas-phase reactivity to the amount of electrophile in the sample.

Calibration Studies of Allyl Halides. Selected reaction monitoring (SRM) calibration curves obtained for the reaction of allyl chloride and allyl iodide with the radical molecular ion of pyridine were linear over approximately 3 decades (slope (m) = 0.87 and 0.91 for allyl chloride and allyl iodide, respectively; Figure 5). Quantities above 5 ng (≈100 pmol) saturated the data system's peak area summation algorithms. Reproducibility, calculated as the average relative standard deviation (RSD), of the response for the allyl chloride and allyl iodide reaction product ions (m/z 120) was ±6.0% for both compounds over the linear portion of the calibration curves. The positive offset (i.e., higher response) of the calibration curve of allyl iodide relative to that of allyl chloride

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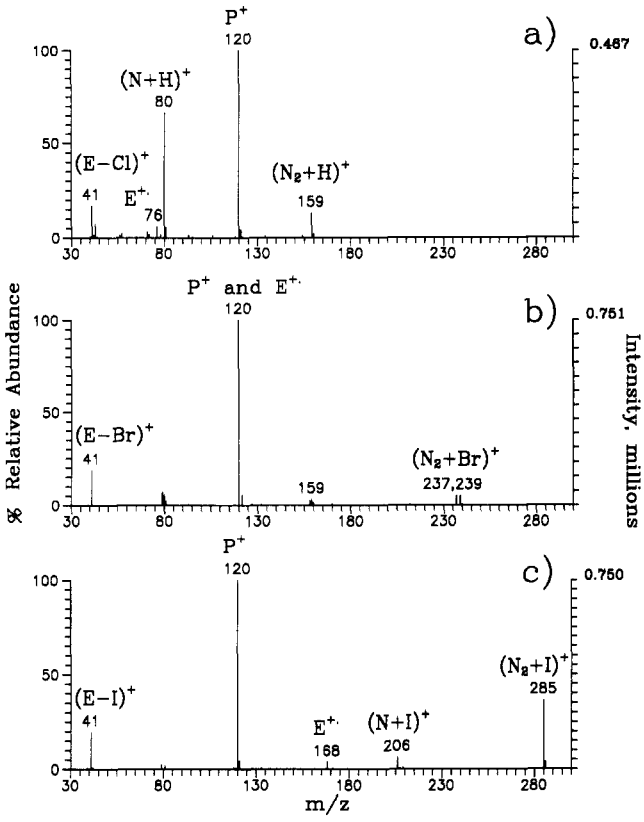

Figure 4. Background-subtracted mass spectra obtained by the reaction of the $CH_4^{\cdot+}$ ion of methane with (a) allyl chloride, (b) allyl bromide, and (c) allyl iodide.

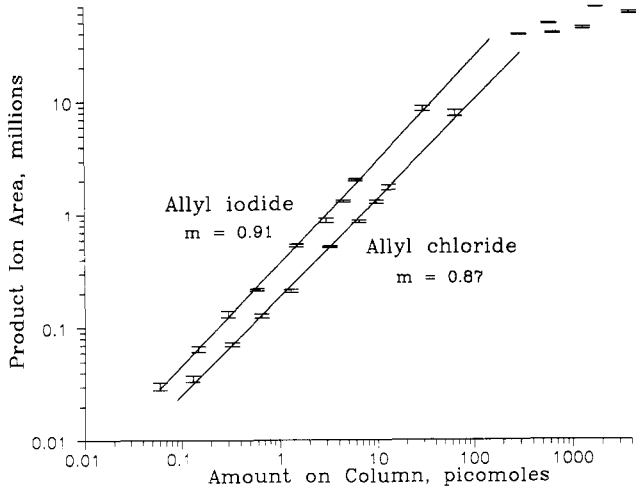

Figure 5. SRM calibration curves obtained for the reaction of the $N^{\cdot+}$ ion of pyridine with allyl chloride and allyl iodide. The error bars correspond to ±1 standard deviation for triplicate injections.

is indicative of the greater gas-phase reactivity of allyl iodide relative to allyl chloride.

Low picogram (≈100 fmol) limits of detection (LOD) were obtained for the product ions (P^+) of these two allyl halides. Figure 6 illustrates the SRM ($N^{\cdot+} \rightarrow P^+$, m/z 120) mass chromatogram obtained for the reaction of 25 pg of allyl chloride (≈330 fmol) and allyl iodide (≈150 fmol). Peaks due to allyl chloride (peak 2) and allyl iodide (peak 4) had a signal-to-noise ratio (S/N) of approximately 10:1. Peaks labeled 1 and 3 are due to the elution of pentane (solvent) and cyclopentane (a solvent impurity), respectively. Neither pentane nor cyclopentane reacted with pyridine $N^{\cdot+}$ ions to

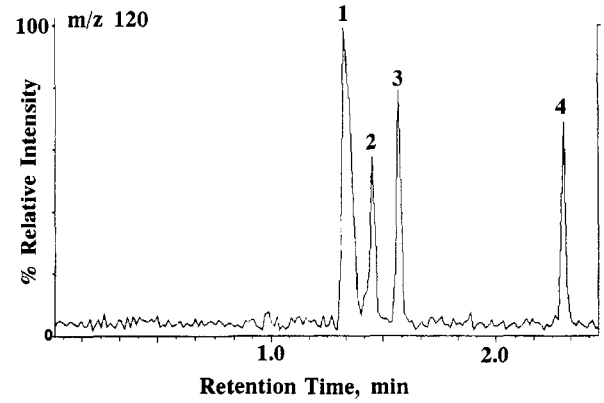

Figure 6. SRM mass chromatogram obtained by the reaction of the $N^{\cdot+}$ ion of pyridine with 25 pg of allyl chloride (2) and allyl iodide (4). The peaks labeled (1) and (3) are due to the elution of pentane (solvent) and cyclopentane (impurity) into the collision cell.

Table 2. Comparison of Gas-Phase $N^{\cdot+}$ Ion Reactivity, Ames Mutagenicity Test, and NBP Alkylating Test

	gas-phase reactivity[a]	mutagenicity[b]	alkylating activity[b,c]
propyl chloride	0.02 (RIC)[e]	0	0
propyl bromide	0.03 (RIC)	NR[f]	NR
allyl isothiocyanate	1.0 (120)[d]	1	1
allyl chloride	2.3 (120)	9	10
2,3-dichloropropene		2.0 (154, 156)	26
benzyl chloride	0.5 (170)	560	98
allyl bromide	3.1 (120)	700	1800
allyl iodide	5.3 (120)	2000	5467

[a] Product ion areas for equimolar solutions, normalized to allyl isothiocyanate reactivity = 1.0. [b] Mutagenicity in revertants per micromole without metabolic activation; alkylating activity defined as the extinction coefficient at 560 nm; ref 6. [c] No detectable chromatographic peak, so baseline noise of the RIC is measured. [d] m/z of product ion measured. [e] Reported values normalized to allyl isothiocyanate. [f] NR, not reported in literature.

form m/z 120 ions. However, the introduction of large amounts of neutrals (e.g., solvent) resulted in an increase in the overall noise level at every m/z, thus producing an apparent peak in the SRM chromatogram.

Relative Gas-Phase Reactivity vs Solution-Phase Reactivity of Neutral Electrophiles. Eder *et al.* have previously determined the mutagenicity and alkylating activity of a series of allylic and halogenated compounds.[6,15] Mutagenicity was predicted by the Ames bacteriological test,[2,16] and alkylating activity was determined with 4-(*p*-nitrobenzyl)pyridine (NBP). The authors reported a nearly quantitative correlation between mutagenicity and alkylating activity for the selected compounds. Table 2 compares the gas-phase reactivity of the pyridine $N^{\cdot+}$ ion with equimolar amounts of eight test compounds to their solution-phase reactivity determined by the NBP alkylating test and the Ames test. The gas-phase reactivity was defined as the area of the product ion peak normalized to the area of the allyl isothiocyanate product ion peak. The propyl halides, which were not mutagenic or alkylating, were not reactive in the gas-phase with pyridine. The reactivity of benzyl chloride was low relative to the Ames test and NBP reactivity. The benzyl chloride reaction was dominated by charge exchange to yield abundant $E^{\cdot+}$ and [E

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– Cl]$^+$ ions with only 2.5 percent of the product ions being P$^+$ ions. The 2,3-dichloropropene was less reactive in the gas phase and in the NBP test than allyl chloride but more mutagenic by the Ames test.[6,15] Eders *et al.* attributed the difference in alkylating activity and mutagenicity for 2,3-dichloropropene to probable metabolic activation during the incubation of the Ames test. They found that the mutagenicity of this compound increased significantly upon metabolic activation with rat liver microsomes. Activation may have occurred also without liver microsomes by either bacterial enzyme activity or by other components of the bacterial suspension. Eder et al. also noted that allyl iodide demonstrated stronger alkylating activity than mutagenicity due to the formation of highly toxic iodine during incubation of the bacteria.

With the exception of benzyl chloride, the gas-phase reactivity of a compound increased with increasing Ames test mutagenicity and with NBP alkylating activity. Thus, this result illustrates the potential of using the gas-phase reactivity with pyridine N$^{\cdot+}$ as an indicator of a compound's propensity to be mutagenic or carcinogenic. However, the range of gas-phase reactivities was not nearly as great as the magnitude of the mutagenicity or alkylating activity. Therefore, compounds with close mutagenicities or alkylating activity, may begin to merge together on the gas-phase reactivity.

In Table 2, the ratio of the allyl iodide product ion to the allyl chloride product ion is 2.3, while in Figure 3, the ratio is 8.8. This difference is attributed to the collision energy applied during the experiments: 2 and 4 eV in Table 2 and Figure 3, respectively. Collision energy studies have shown that the allyl iodide product ion is readily formed at collision energies ranging from 2 to 8 eV while the allyl bromide and allyl chloride product ions begin to decrease dramatically at collision energies above 2 eV.[17]

Mass-Selected Ionized Electrophile/Neutral Nucleophile Reactions in Collision Cell. Reactions of the radical molecular ion of environmental contaminants with a neutral nucleophilic reagent require that the reactive ion be known so that it can be selected for reaction by Q1. Although less analytically useful for screening complex samples, it can be highly effective for screening for a specific analyte. Therefore, reactions of radical molecular ions and fragment ions of allylic reagents with neutral pyridine in the collision cell were also studied.

Relative Reactivity of Ionized Electrophiles. In the determination of the relative reactivity of neutral electrophiles with pyridine ions, equimolar solutions were utilized and the direct comparison of product ion peak areas yielded the relative reactivities. Although equimolar solutions were utilized in these studies also, the comparison of the product ion (P$^+$) intensities was complicated by the fact that the intensities of the mass-selected E$^{\cdot+}$ ions were not equivalent for each analyte. Fragmentation of the ions in the ion source and the distribution of isotope ions changed the intensity of the reaction ion. To account for this, a normal Q1 mass spectrum is obtained alternately with a mass-selected reaction product spectrum. Since pyridine is present in the collision cell during the Q1 MS scan, the ions of the electrophiles entering the collision cell can still undergo reactions with pyridine. However, as no

(17) Freeman, J. A. Ph.D. Dissertation, University of Florida, May 1991.

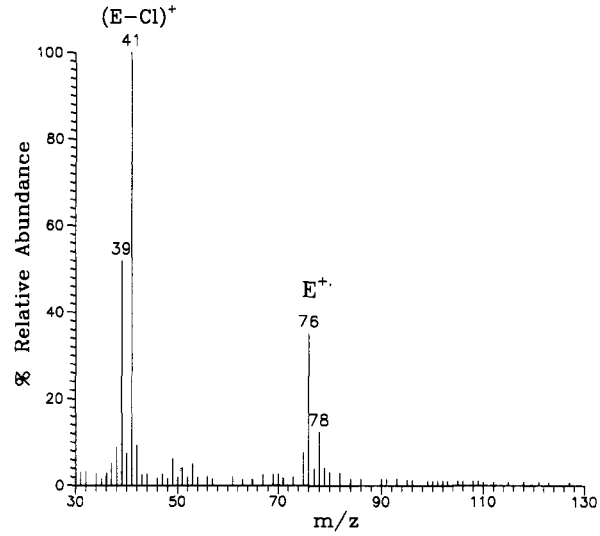

Figure 7. Q1MS mass spectrum of allyl chloride obtained with neutral pyridine in the collision cell.

mass-selection is being performed by Q3, all ions are transmitted through Q2, and the ion current detected is assigned a m/z as determined by the m/z being transmitted by Q1. Thus, a normal mass spectrum is obtained as illustrated in Figure 7 for allyl chloride. This mass spectrum contains the same ions with similar relative abundances (as well as similar absolute abundance) as the Q1 mass spectrum obtained without pyridine in the collision cell. Thus, the yield of electrophile reactant ions resulting from ionization and isotope distribution can be determined. Note that for the case of unknowns, a Q1 mass spectrum must be obtained first in order to determine what ions to mass-select and when (i.e., the retention time) to mass-select them for reaction with pyridine.

The reactivities of the E$^{\cdot+}$ and [E – X]$^+$ ions, produced under EI conditions, and [E + H]$^+$, [E + C_2H_5]$^+$, [E + C_3H_5]$^+$, and [E – H]$^-$ ions, produced under CI conditions, for allyl isothiocyanate, allyl chloride, allyl bromide, and allyl iodide with neutral pyridine in the collision cell were determined. Each positive ion produced the associative product ion at m/z 120 (P$^+$) in varying intensities. Since the ions produced under CI conditions have potential hydrogen-bonding sites, in addition to the 120$^+$ product ion, several hydrogen-bonded adduct ions were observed. The [E + H]$^+$ ions reacted to produce mainly [E + H + N]$^+$ ions (N = nucleophile pyridine) and some [P + H]$^+$ ions while the [E + C_2H_5]$^+$ and [E + C_3H_5]$^+$ ions reacted to produce the [E + C_2H_5 + N]$^+$ and [E + C_3H_5 + N]$^+$ ions, respectively. No detectable reaction product was observed when the [E – H]$^-$ ion was selected for any of the allylic compounds.

The mass spectra shown in Figure 8 were obtained by reaction of neutral pyridine with the E$^{\cdot+}$ ions of allyl chloride (^{35}Cl isotope, m/z 76), allyl bromide (^{81}Br isotope, m/z 122), and allyl iodide (m/z 168). The ^{81}Br E$^{\cdot+}$ ion (m/z 122) of allyl bromide was chosen rather than the ^{79}Br E$^{\cdot+}$ (m/z 120) in order to eliminate the possibility of the m/z 120 in the product spectrum being a combination of the P$^+$ and E$^{\cdot+}$ ions. The product ion (P$^+$) was observed at m/z 120 for each allylic electrophile. In addition to product ion formation, there was abundant protonated pyridine (i.e., [N + H]$^+$ ions) at m/z 80. Although pyridine could have abstracted a proton directly

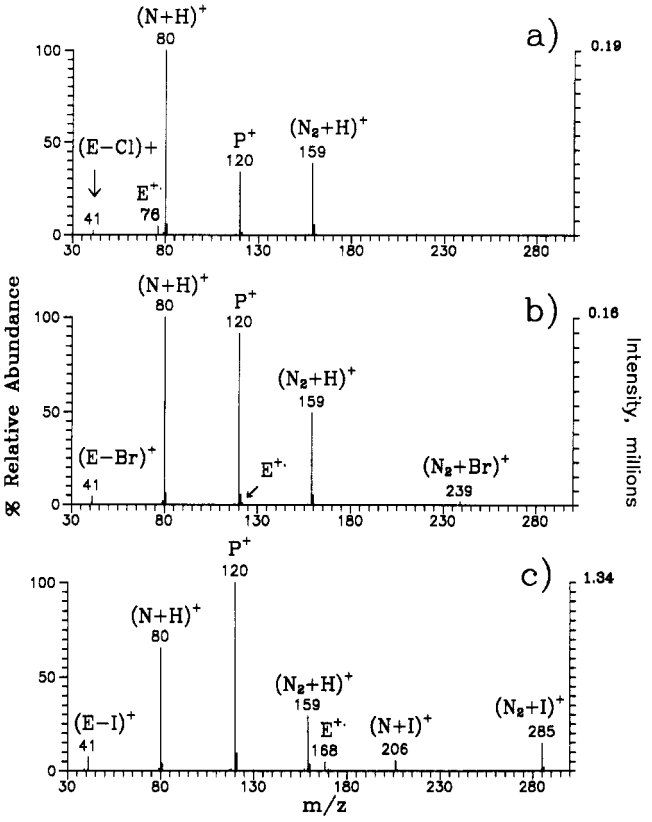

Figure 8. Mass spectra obtained by the reaction of neutral pyridine with the $E^{\cdot+}$ ion of (a) allyl chloride, (b) allyl bromide, and (c) allyl iodide.

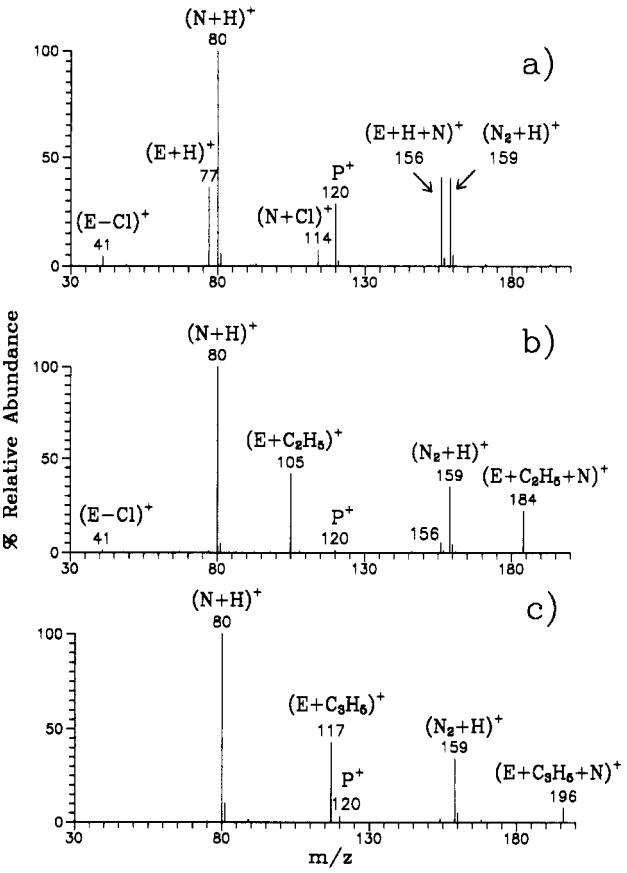

Figure 9. Mass spectra obtained by the reaction of neutral pyridine in the collision cell with the mass-selected (a) $[E + H]^+$, (b) $[E + C_2H_5]^+$, and (c) $[E + C_3H_5]^+$ ions of allyl chloride.

from the $E^{\cdot+}$ ions, it is more likely that $[N + H]^+$ is formed from the fragmentation of the product ion $P^{\cdot+}$ This latter mechanism is supported by tandem mass spectrometric experiments on a quadrupole ion trap: the product ion was produced by mass-selected reaction of the $E^{\cdot+}$ ion of allyl chloride and neutral pyridine and then fragmented by collisionally activated decomposition (CAD) to produce largely the $[N + H]^+$ and $C_3H_5^+$ ions.[18] In addition, some of the $[N + H]^+$ ions may have originated from charge-exchange reactions as indicated in the following paragraph. This $[N + H]^+$ ion produced in the collision cell then reacted with neutral pyridine to produce the proton-bound dimer of pyridine ($[N_2 + H]^+$) at m/z 159, which was observed in the mass spectra of each analyte (Figure 8). The fragment ion $[E - X]^+$ at m/z 41, produced by CAD of $E^{\cdot+}$ and fragmentation of the P^+ ion, was also observed in each of the mass spectra. The ion observed at m/z 239 in the allyl bromide reaction spectrum is thought to be $[N_2 + Br]^+$ and was also observed in the spectra of the reaction of neutral allyl bromide with ionized pyridine (Figure 3b). The $[N + I]^+$ and $[N_2 + I]^+$ ions were observed in the mass spectrum obtained by reaction of the allyl iodide $E^{\cdot+}$ ion; however, no corresponding ions were observed for the reaction of the allyl chloride $E^{\cdot+}$ ion.

Since each of the allylic reagents has an ionization energy (IE) greater than or nearly equal to that of pyridine, allylic $E^{\cdot+}$ ions can undergo charge exchange reactions with neutral pyridine to produce $N^{\cdot+}$. This accounts for the small amount of m/z 79 observed in each mass spectra of Figure 8. Further reaction of $N^{\cdot+}$ with neutral pyridine may have contributed to the $[N + H]^+$ intensity also. In addition, since only small quantities of allylic reagents were introduced into the ion source, few neutral electrophile molecules would be expected to enter the collision cell. Therefore, the $[N^{\cdot+} + E \rightarrow P^+]$ side reactions, which could occur during the $E^{\cdot+}/N$ reactions, would be expected to form only very small amounts of m/z 120 product ion. This is in contrast to the case when much larger quantities of pyridine are leaked into the source for ionized nucleophile/neutral electrophile reactions.

Mass spectra of the reaction of neutral pyridine with the ^{35}Cl isotope of $[E + H]^+$, $[E + C_2H_5]^+$, and $[E + C_3H_5]^+$ ions of allyl chloride are shown in Figure 9. Although all of these reactions resulted in the formation of some m/z 120 product ions (P^+), overall yield was much lower than for reactions with $E^{\cdot+}$. Rather, reaction products are dominated by proton transfer to pyridine to produce $[N + H]^+$ ions that subsequently react with pyridine to form abundant $[N_2 + H]^+$ ions. Other abundant product ions are due to adduct formation between the mass-selected electrophile ion and neutral pyridine, i.e., $[E + H + N]^+$, $[E + C_2H_5 + N]^+$, and $[E + C_3H_5 + N]^+$ ions.

Calibration Studies of Allyl Halides. Calibration curves obtained for reactions of the radical molecular ions of allyl chloride and allyl iodide with neutral pyridine in the selected reaction monitoring (SRM) mode are shown in Figure 10. Product yield was linear over approximately 3 decades (slope (m) = 0.96 and 0.95 for allyl chloride and allyl iodide, respectively). Calibration was nonlinear above ≈1000 pmol due to limitations of the data system summation algorithms.

(18) Johnson, J. V. Unpublished data, February 1991.

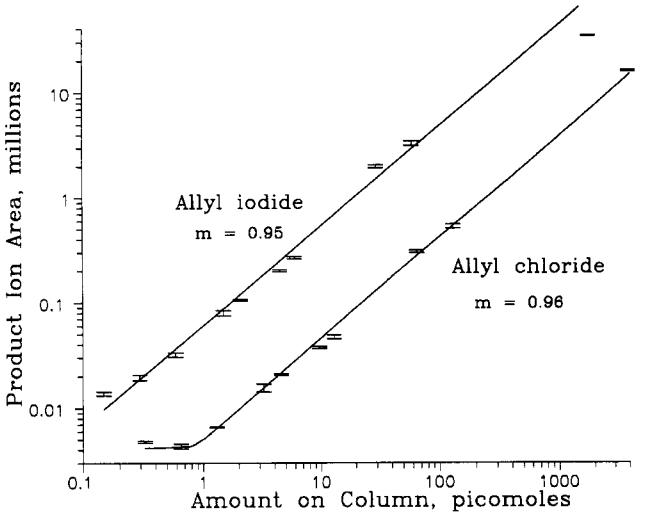

Figure 10. SRM calibration curves obtained by the reaction of the $E^{\cdot+}$ ion of allyl chloride and allyl iodide with neutral pyridine in the collision cell. The error bars correspond to ±1 standard deviation for triplicate injections.

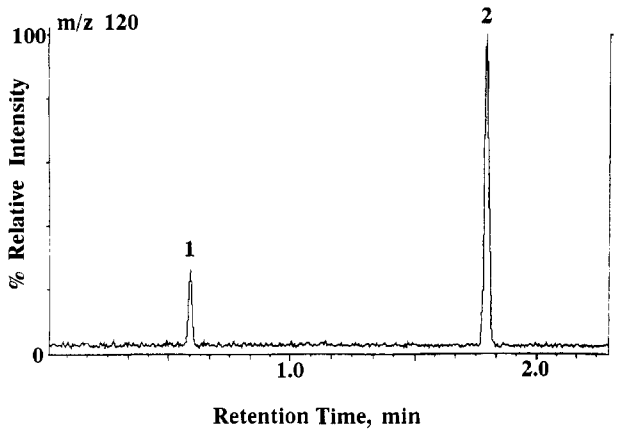

Figure 11. SRM mass chromatogram (*m/z* 120) obtained for reaction of the $E^{\cdot+}$ ions of 25 pg each of allyl chloride (1) and allyl iodide (2) solution with neutral pyridine in the collision cell. The signal/noise ratios were 6.0 and 23 for allyl chloride and allyl iodide, respectively.

Reaction product peak areas for allyl chloride and allyl iodide had a relative standard deviation of ±6.0% and ±6.4%, respectively, indicating good reproducibility. Positive offset of the calibration curve for allyl iodide relative to that for allyl chloride was due to the difference in yield of $E^{\cdot+}$ ions during ionization, mass-selection of only ^{35}Cl $E^{\cdot+}$ ions of allyl chloride, and greater gas-phase reactivity of $E^{\cdot+}$ ions of allyl iodide than allyl chloride. Low picogram (≈100 fmol) limits of detection (LOD) were obtained for product ions of these two allyl halides (Figure 11). Mass chromatograms for 25 pg each of the two allyl halides (Figure 11) show a S/N of ≈6 for allyl chloride (≈330 fmol) and ≈23 for allyl iodide (≈150 fmol). In contrast to the ionized nucleophile SRM chromatograms of Figure 6, Figure 11 shows only peaks due to the allyl halides. The solvent and its impurities do not yield a response due to the differential pumping of the source and analyzer region and as no solvent ions were mass-selected for reactions.

Calibration curves obtained for reactions of allyl chloride and allyl iodide radical molecular ions with neutral pyridine in the full scan mode (Q3 scanned from 30 to 280 amu) are shown in Figure 12. Product yield was linear over ≈2 decades

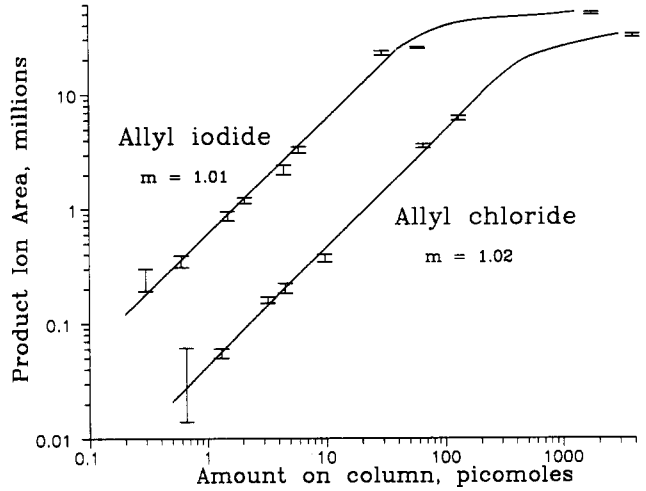

Figure 12. Full scan calibration curves obtained by the reaction of the $E^{\cdot+}$ ion of allyl chloride and allyl iodide with neutral pyridine in the collision cell. The error bars correspond to ±1 standard deviation for triplicate injections.

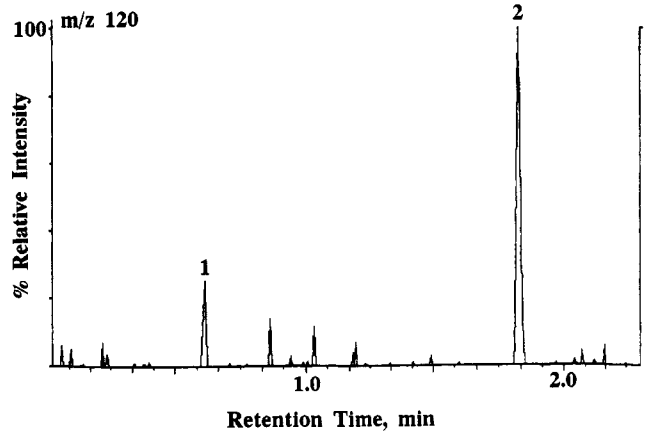

Figure 13. Full scan mass chromatogram (*m/z* 120) obtained for the reaction of the $E^{\cdot+}$ ions of 100 ng each of allyl chloride (1) and allyl iodide (2) solution with neutral pyridine in the collision cell.

(*m* = 1.02 and 1.01 for allyl chloride and allyl iodide, respectively) and had an upper limit of 100 pmol. Peak area reproducibility was ±10.9% RSD for both compounds. The LOD was ≈100 pg (≈1 pmol at S/N ratios of ≈2 for allyl chloride (1.3 pmol) and ≈10 for allyl iodide (0.6 pmol) (Figure 13).

Gas-Phase vs Solution-Phase Reactivity. The gas-phase reactivity of ionized allyl isothiocyanate, allyl chloride, allyl bromide, and allyl iodide was determined and compared with Ames test mutagenicity[6,15] (Table 3). Only $E^{\cdot+}$ ion reactivity was comparable to Ames test results in that the order of gas-phase reactivity and mutagenicity was allyl iodide > allyl bromide > allyl chloride > allyl isothiocyanate. Since the allyl $[E-X]^+$ ion has the same composition ($C_3H_5^+$) for each of these four allyl compounds, gas-phase reactivity was expected to be nearly the same. Indeed, the reactivity of $[E-X]^+$ from each allyl halide was equivalent. However, the reactivity of $[E-X]^+$ from allyl isothiocyanate was significantly lower, possibly due to lower internal energy of a fragment ion resulting from loss of a polyatomic group. The gas-phase reactivities of the $[E+H]^+$ ions were much lower than those of the $E^{\cdot+}$ and $[E-X]^+$ ions with respect to the *m/z* 120 product ion. However, the $[E+H]^+$ ions react to

Table 3. Comparison of Gas-Phase Reactivity of Various Ionized Electrophiles and Ames Mutagenicity Test

electrophile	mass-selected electrophile ions $E^{\cdot+}$	mass-selected electrophile ions $[E-X]^+$	$[E+H]^+$		mutagenicity[b]
allyl isothiocyanate	1.0[a]	1.0[a]	1.0[a]	1.0[a]	1
allyl chloride	4.8	2.1	1.2	1.5	9
allyl bromide	8.8	2.0	0.5	1.6	700
allyl iodide	16.0	2.0	1.8	1.9	2000

[a] Product ion (120^+) areas/Q1MS electrophile ion area, normalized to allyl isothiocyanate = 1.0. [b] Mutagenicity in revertants per microliter without metabolic activation; ref 6. [c] $\Sigma(P^+ + [E + H + N]^+)$ ion areas/Q1MS electrophile ion area, normalized to allyl isothiocyanate = 1.0.

Table 4. Comparison of Gas-Phase $E^{\cdot+}$ Ion Reactivity to Ames Mutagenicity Test and NBP Alkylating Test

electrophile	$E^{\cdot+}$ (m/z)[a]	gas-phase reactivity[b]	mutagenicity[c]	alkylating activity[c,f]
propyl chloride	78	0.001 (RIC)[d]	0	0
propyl bromide	128	0.001 (RIC)	ND[g]	ND
allyl isothiocyanate	99	1.0 (120)[e]	1	1
allyl chloride	76	4.8 (120)	9	10
2,3-dichloropropene	ND[h]	ND	26	8
benzyl chloride	126	0.5 (170)	560	98
allyl bromide	122	8.8 (120)	700	1800
allyl iodide	168	16.1 (120)	2000	5467

[a] Electrophile ion mass-selected with Q1. [b] Product ion areas/Q1MS $E^{\cdot+}$ ion areas for equimolar solutions normalized to allyl isothiocycnate reactivity. [c] Mutagenicity in rev/μmole without metabolic activation; alkylating activity defined as the extinction coefficient at 560 nm; ref 6. [d] No detectable peaks, so baseline noise of the RIC is measured. [e] m/z of product ion measured. [f] Reported values normalized to allyl isothiocyanate. [g] ND, not determined.

yield abundant $[E + H + N]^+$ ions. Consideration of both product ions triples the allyl bromide reactivity, with very little effect on any of the other allyl compounds. Note that there is very little range in the reactivities (1–1.9) of the $[E + H]^+$ ions. Due the low yields of the m/z 120 product ions, attempts to quantitate the reactivity of the $[E + C_2H_5]^+$ and $[E + C_3H_5]^+$ offer little insight into their relative reactivities.

Table 4 compares the gas-phase reactivities of the $E^{\cdot+}$ ion of a series of allyl compounds and two propyl compounds to their solution-phase reactivities determined by the NBP alkylating test and the Ames bacteriological test. The data were obtained by alternating between mass-selecting the $E^{\cdot+}$ ion of the electrophile and a Q1MS scan; thus, the product ions are normalized to the amount of $E^{\cdot+}$ ion produced in the ion source (i.e., isotopic patterns are corrected also). Similar results were obtained from ionized nucleophile/neutral electrophile reactions. Compounds mutagenic in the solution-phase tests were reactive in the gas phase. The $E^{\cdot+}$ ions of propyl chloride and propyl bromide were nonreactive in the gas phase. 2,3-Dichloropropene was not tested. Although the trend of the relative gas-phase reactivities was similar to that obtained for the ionized nucleophile/neutral electrophiles (Table 2), the range of the relative gas-phase reactivities of the $E^{\cdot+}$ ions (0.5–16.1, Table 4) was approximately three times that of the $N^{\cdot+}$ ions (0.5–5.3, Table 2).

CONCLUSIONS

Gas-phase ion–molecule reactions have been investigated as a unique tool for the characterization of potentially genotoxic (electrophilic) chemicals in complex environmental samples. The ability to produce and detect associative reaction ion products formed in the collision cell of a tandem quadrupole mass spectrometer by the reaction of a nucleophilic compound (pyridine; a model for DNA) with electrophilic environmental contaminants is demonstrated. Associative reaction product spectra are simple, and product ion detection is highly sensitive. Two modes of reaction are possible: (1) ionization of the nucleophilic reagent followed by reactions with neutral electrophilic chemicals in the collision cell and (2) ionization of the electrophilic environmental contaminant followed by reactions with neutral nucleophilic reagent in the collision cell. The investigation of the reaction of nucleophilic pyridine with a series of allylic reagents shows that the relative gas-phase reactivity of the radical molecular ion of either reagent with the neutral molecule of the other correlates with the electrophiles' relative alkylating activity in solution and Ames test mutagenicity, previously reported in the literature.

Low picogram (≈100 fmol) limits of detection are obtained in the selected reaction-monitoring mode for ionized electrophile and ionized nucleophile reactions; ≈5 times higher limits of detection are obtained for wide mass range product ion scans. Compared to the Ames test in which mutagens are detected in low microgram or in some cases in nanogram amounts,[18] the detection limits obtained by this method are better by orders of magnitude and are adequate to detect potential genotoxic compounds in environmental mixtures. Furthermore, the sensitivity and rapidity of the response make it possible to characterize individual components as they elute from a GC column.

The investigations carried out in this paper encompass a relatively small set of well-characterized mutagens. Further studies are necessary to assess the capability of these ion–molecule reactions to detect other classes of potential biomolecule alkylating agents.

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