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Reduction of Aryl Halides and *N*-Hydroxy Nitrogen Compounds in Fast Atom Bombardment Mass Spectrometry

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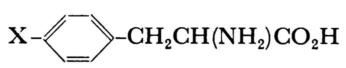
Aromatic halogenated compounds and *N*-hydroxy nitrogen compounds were reduced to dehalogenated and deoxy analogs, respectively, in the fast atom bombardment ion source of a mass spectrometer. The reaction was found to occur in the liquid phase on exposure to a neutral fast atom beam.

Fast atom bombardment mass spectrometry (FAB/MS) and mass spectrometry mass spectrometry (MS/MS)[1,2] are very useful tools for characterizing polar or nonvolatile organic compounds.[3,4] In connection with a study on the FAB/MS of naturally occurring chlorinated peptide antibiotics (*e.g.* vancomycin[5,6]), we found that ions which might be assigned to quasi-molecular ions of the original compounds and those of mono-dechlorinated analogs were observed at the same time. These dechlorinated species seemed to exist regardless of the purity of the original samples. So this phenomenon was considered to be attributable to reduction of the corresponding chlorinated compounds. To confirm the assumption, halogenated model compounds were examined. Furthermore, several *N*-hydroxy nitrogen compounds were also tested for reduction under FAB measurement conditions.

Results and Discussion

The FAB spectrum of *p*-chlorophenylalanine (**1**) is shown in Fig. 1. A protonated molecular ion (*m/z* 200) was observed in the spectrum. Surprisingly, a non-chlorine-containing ion (*m/z* 166) corresponding to MH$^+$ of phenylalanine was also recorded at a notable relative intensity (23% of the sum of *m/z* 200 and 202). This ion (*m/z* 166) can not be derived from MH$^+$ of **1** (*m/z* 200) since mass difference between these ions (34 u) is smaller than mass number of chlorine. The structure of the ion at *m/z* 166 was determined to be the same as that of MH$^+$ of phenylalanine, because the collisionally activated dissociation (CAD) spectra of both ions were identical (Fig. 2).



1, X=Cl
2, X=F
3, X=Br

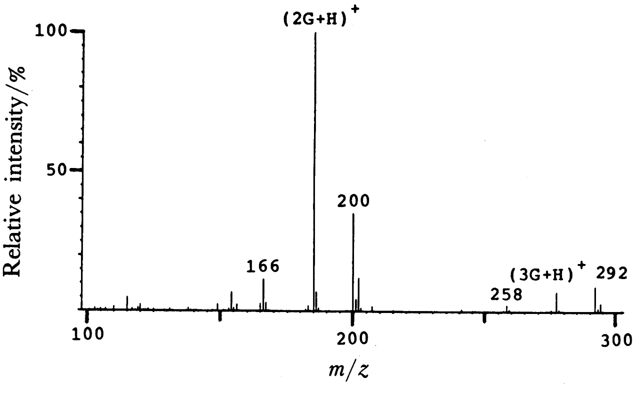


Fig. 1. Positive ion FAB spectrum of **1**. Glycerol is represented by G. The ions at *m/z* 258 and 292 are glycerol adducts of *m/z* 166 and 200, respectively.

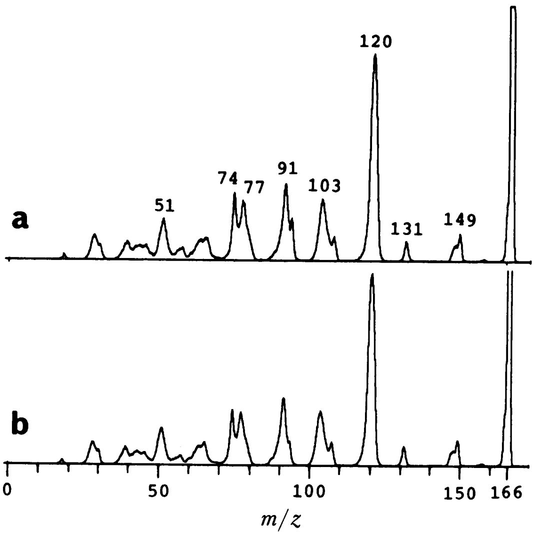


Fig. 2. CAD spectra of (a) *m/z* 166 in the FAB spectrum of **1** and (b) protonated molecular ion of phenylalanine yielded by FAB ionization. The precursor ions were resolved at high resolution (10,000).

The relative abundance of *m/z* 166 was dependent on the kinetic energy of the neutral fast atom beam (16, 23, and 26% for 4, 6, and 8 keV, respectively) and increased over the period of irradiation (Fig. 3). These results suggest that the ion at *m/z* 166 was formed by bombardment of the fast atom beam at the surface of the sample solution.

The FAB spectra of *p*-fluorophenylalanine (**2**) and *p*-bromophenylalanine (**3**) also showed identical ions (*m/z* 166). The relative abundances of the ions at *m/z* 166 were 3.3% to *m/z* 186 and 51% to the sum of *m/z* 244 and 246, respectively. It seemed that the carbon–halogen bond strength directly reflected the amount of the reduced species.

Very recently, similar results on halogenated nucleosides have been reported by McCloskey *et al.*[7] They proposed the process that the halogen atoms on aromatic rings were replaced by hydrogen atoms of

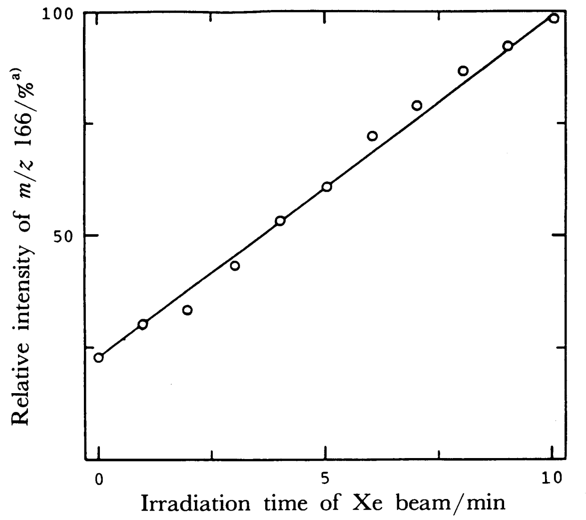
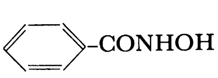


Fig. 3. Relationship between irradiation time and relative abundances of *m/z* 166 in FAB spectra of **1**. a) Calculated based on the sum of *m/z* 200 and 202.

glycerol molecules *via* radical intermediates based on deuterium labeling experiments. In present MS/MS studies, on the other hand, the structure of reduced products of **1**—**3** have been confirmed directly.

Such reductions occur not only in the case of halogenated compounds. Other examples were found in the case of *N*-hydroxy nitrogen compounds, namely, benzohydroxamic acid (**4**), 1-hydroxybenzotriazole (**5**), and *N*-hydroxysuccinimide (**6**).



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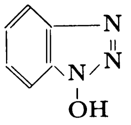

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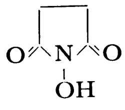

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The FAB spectrum of **4** (Fig. 4) exhibits MH^+ of **4** (*m/z* 138) and $MH^+ -16$ (*m/z* 122, 60% of *m/z* 138). The ion at *m/z* 122 was not derived from MH^+ (*m/z* 138), because the metastable ion (MI) spectrum and CAD spectrum of MH^+ did not show any peak at *m/z* 122. The CAD spectrum of *m/z* 122 is identical to that of MH^+ of benzamide (Fig. 5). These results indicate that **4** was reduced to benzamide during the measurement of the FAB spectrum.

Similar phenomena were observed in the cases of **5** and **6**. The FAB spectra of **5** and **6** showed *m/z* 120 ($MH^+ -16$, 51% of MH^+) and *m/z* 100 ($MH^+ -16$, 31% of MH^+), respectively. The MS/MS experiments revealed that the ions ($MH^+ -16$) were not derived from MH^+ but were identical to MH^+ of the corresponding reduced products in each case.

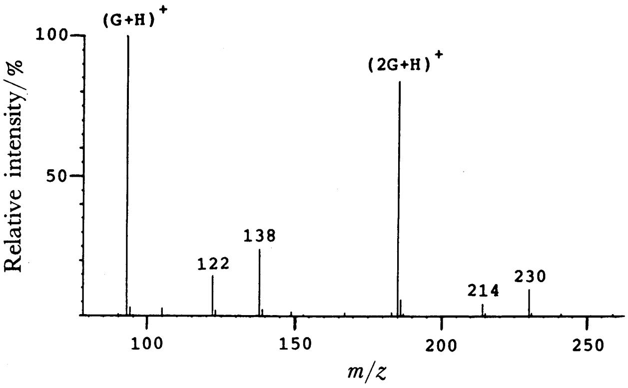

Fig. 4. Positive ion FAB spectrum of **4**. Glycerol is represented by G. The ions at *m/z* 214 and 230 are glycerol adducts of *m/z* 122 and 138, respectively.

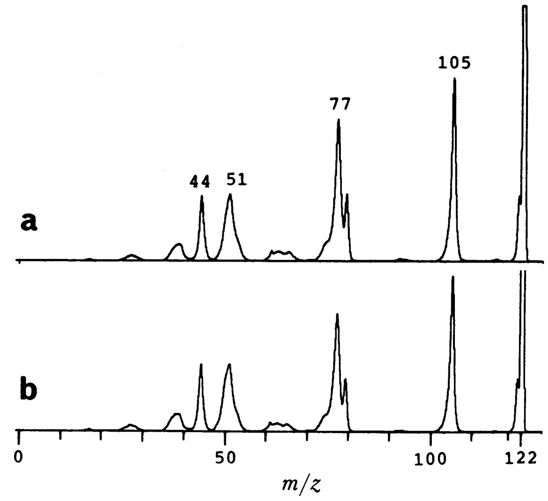

Fig. 5. CAD spectra of (a) *m/z* 122 in the FAB spectrum of **4** and (b) protonated molecular ion of benzamide yielded by FAB ionization. The precursor ions were resolved at high resolution (10,000).

Since trace amounts of reduced products were recovered from the sample matrix solutions after FAB measurement of compounds **1**, **3**, **4**, and **5**, it was determined that the reactions occurred in the liquid phase as a result of bombardment by fast atoms, at least in part. Accumulation and time dependence of the relative intensities of MH^+ of the reduced products were not observed in previous work.[7] This disagreement may be caused by some differences of experimental conditions, *e.g.*, sample concentration, density of neutral atom beams, sputtering efficiency of sample molecules, and pressure of ion sources.

The results presented in this paper indicate that it is difficult to determine the purity or quantity of the above types of compounds by FAB/MS. However, in the cases that the purity of the samples has been established on the basis of other data, the phenomena described above are very useful for characterization of these compounds.

Experimental

Materials. All compounds were obtained from commercial sources: **1**, **2**, **4**, **5**, and **6**, Tokyo Kasei Kogyo Co.; **3** and glycerol (99.5%), Aldrich Chemical Co. They were used without further purification. Compounds **1**—**6** were examined by GC/MS as trimethylsilyl derivatives (for **4**—**6**) or *N*-trifluoroacetyl trimethylsilyl esters (for **1**—**3**). Dehalogenated analogs of **1**—**3** or *N*-deoxy analogs of **4**—**6** could not be detected in each case (the limits of detection were 0.25% for dehalogenated analogs and 1% for deoxy analogs). Xenon (99.998%) was purchased from Showa Denko Co.

Apparatus and Procedures. All FAB mass spectra and MS/MS (MI and CAD) spectra were obtained using a JEOL JMS-HX100 mass spectrometer, which consists of a conventional geometry high resolution mass spectrometer followed by a second electrostatic analyzer (ESA-2). The kinetic energy of the xenon fast atom beam was made up to 6 keV unless otherwise stated.

The methanolic or aqueous ethanolic solutions of 10μg samples were mixed with glycerol (1 μl), then applied on stainless steel probe tips and introduced into a FAB ion source through a vacuum lock. Scanning of the magnetic field and irradiation of a fast atom beam were started at the same time and FAB spectra were recorded.

The MI and CAD spectra were obtained with the ESA-2 scanning. Collisionally activated dissociation experiments were performed at a high collision energy (5 keV).[2] Chemically pure argon was used as a neutral target gas introduced into the collision cell placed between the magnetic field and the ESA-2.

Sample solutions were recovered after irradiation by a fast atom beam (*ca.* 30 min) and examined by TLC.

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