

Derivatization of Biomolecules for Analysis by Fast Atom Bombardment Mass Spectrometry

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Introduction

Conventional ionization methods used in mass spectrometry are limited to samples sufficiently volatile to allow vaporization without thermal decomposition. In recent years, several new ionization techniques have been developed that are designed to circumvent this sample volatility requirement. Perhaps the most popular of these new ionization techniques is fast atom bombardment (FAB) mass spectrometry. FAB is now routinely used in the analysis of nonvolatile and thermally labile compounds, and has expanded the scope of the analysis to compounds of biological interest.

The usual procedure of analysis by FAB is dissolution of the sample in a suitable liquid matrix, commonly glycerol, followed by bombardment of the dissolved sample with a 2-7 keV beam of xenon atoms. The ions sputtered from the solution into the gas phase are analyzed to form the FAB mass spectrum. The ionization process involves two steps, (i) the transfer of material from the condensed phase to the gas phase, and (ii) ionization of the sample molecules by chemical processes occurring in the same time frame. Common characteristics of the mass spectra thus obtained include the rarity of odd electron molecular ions, and lower yields of cationized molecules such as $(M+H)^+$ and $(M+Na)^+$ relative to intact cations of ammonium, sulfonium, and other onium salts. This observation indicates that it would be advantageous to convert the sample molecules to an ionic form prior to analysis by FAB. The ionization process would then involve a single step, the sputtering of preformed ions from the sample surface. This single step process is much more efficient than the two step process above. This is confirmed experimentally, as the enhanced signal-to-noise ratio for ions from derivatized molecules decreases detection limits. Enhanced selectivity is also available if the derivatization procedure used is specific for a particular type of functional group.

Methods and Materials

FAB mass spectra were obtained on a Kratos MS8ORFAQQ mass spectrometer of EBQQ geometry, using the intermediate detector. A resolution of 1000 was used to record all spectra. Xenon gas was fed into an Iontech gun to produce neutrals of 7 keV energy. The sample mixture (2 μ L) was loaded onto a copper platform which intercepted the primary beam at an angle of 45 degrees. The spectra shown are not corrected for background.

Steroids, kanamycin sulfate, and 2-fluoro-1-methylpyridinium p-toluenesulfonate were used as received from Sigma Chemical Company. The reagent 2,4,6-trimethylpyrylium tetrafluoroborate was used as received from Alfa Products.

Results and Discussion

One method of sample derivatization is the use of an ion attachment reaction defined as the formation of a bond between the neutral sample molecule and a charge carrier. Two examples of nonspecific ion attachment reactions are the protonation of a sample to form the corresponding $(M+H)^+$ ion, and the attachment of a sodium cation to an analyte to form the $(M+Na)^+$ ion. An abundance of functional-group-specific reactions that produce ionic products can be found in the literature of syn-

thetic organic chemistry and electrophoresis. For example, the reagent 2-fluoro-1-methylpyridinium p-toluenesulfonate reacts with hydroxyl functional groups, one of several functional groups commonly found in biomolecules, to form the corresponding N-pyridinium derivative bound through an ether linkage to R of the ROH analyte (3). This reagent reacts with the steroid corticosterone, leading to the FAB mass spectrum of the corresponding N-pyridinium salt in Figure 1. An abundant intact cation is observed

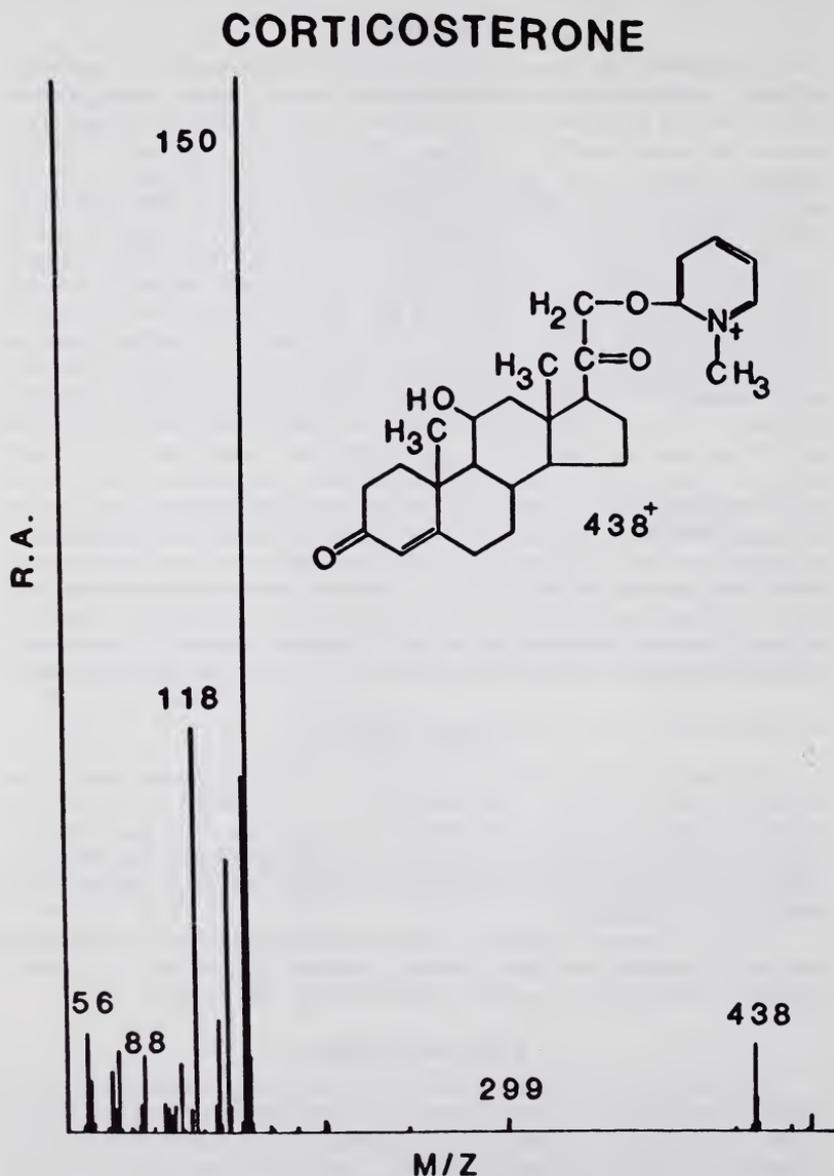


FIGURE 1. FAB mass spectrum of the N-pyridinium derivative of the steroid corticosterone.

at m/z 438 that is well removed from interfering background peaks due to the triethanolamine liquid matrix. The reagent 2,4,6-trimethylpyridinium tetrafluoroborate reacts with primary amine groups, another functional group commonly found in biomolecules, to again form an N-pyridinium salt (4). This derivatization results in the appearance of an abundant signal for the cation in the FAB mass spectrum for the N-pyridinium derivative. The signal appears at m/z ($M + 105$), where M is the molecular weight of the primary amine. For example, Figure 2 is the FAB mass spec-

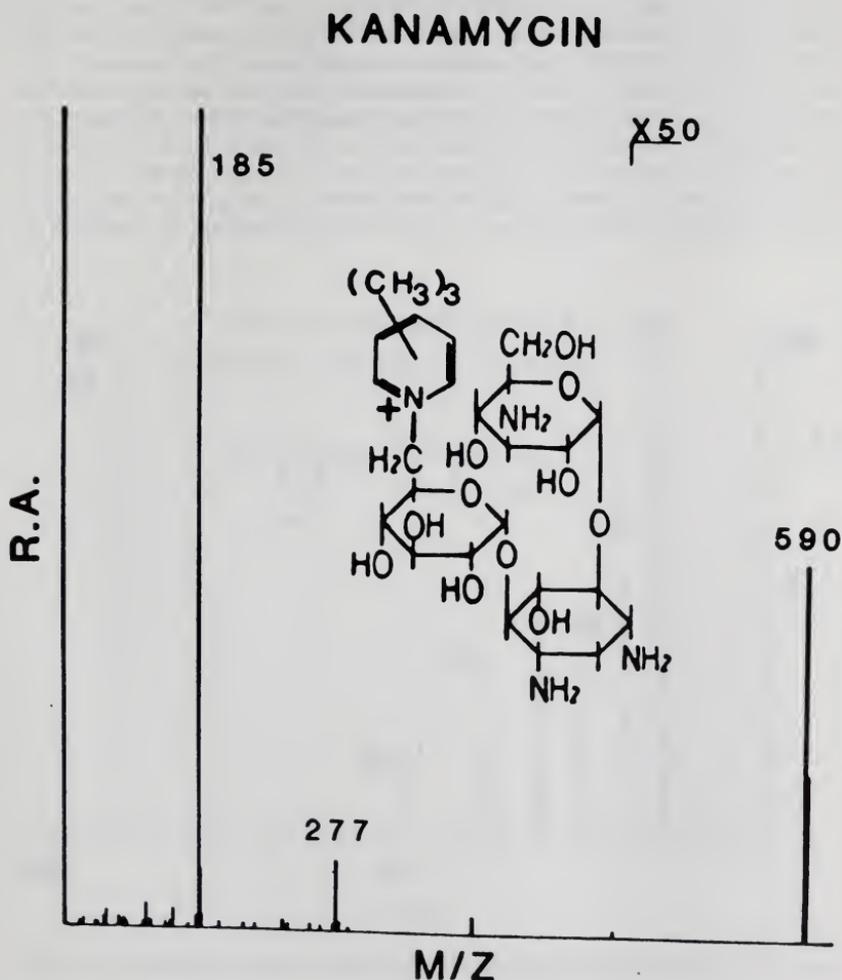


FIGURE 2. FAB mass spectrum of the N-pyridinium derivative of the aminoglycosidic antibiotic kanamycin sulfate.

trum of the derivative of the aminoglycosidic antibiotic kanamycin sulfate. An abundant intact action observed at m/z 590 is the sole abundant ion observed for the sample itself. Also observed are two background peaks at m/z 185 and 277 due to the glycerol matrix.

A second method of derivatization, charge transfer, is also under investigation. Charge transfer derivatization reactions are particularly useful in that the ion formed directly indicates the molecular weight of the sample, and further that many classes of compounds show a unique propensity to react via charge transfer complex formation. When placed in a polar solvent, charge transfer complexes ($D^+ A^-$) consisting of electron donors (D) and electron acceptors (A) undergo dissociation into ions. Thus, charge transfer complexes should produce the radical ions D^+ and A^- in the FAB mass spectrum. However, charge transfer derivatization reactions have been shown to be explicitly solvent dependent (2). As an example, usual FAB solvents, although polar enough to stabilize ion pairs essential to the reaction, also possess acid/base properties which dominate the production of secondary ions. Proton transfer reactions occur both in the gas phase as large sputtered cluster ions undergo desolvation, but also in the condensed phase. As a result of the acid/base properties of the solvent one must make a careful choice of liquid matrix to be used in the FAB analysis of charge transfer complexes. An alternate solution to the solvent-dependence problem is to eliminate the liquid matrix. The FAB mass spectrum of the anthracene-picric acid charge transfer complex is shown in Figure 3 reflects a ten-fold increase in abundance for

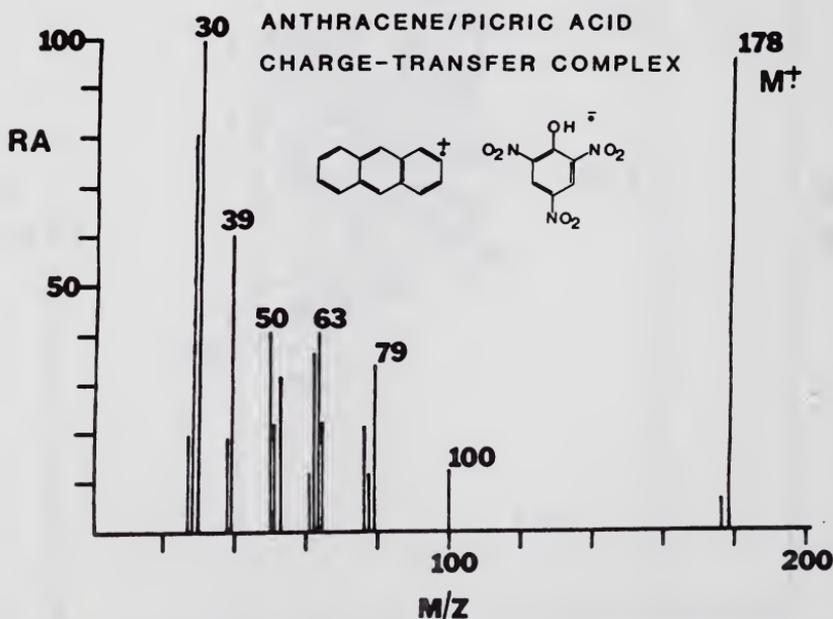


FIGURE 3. FAB mass spectrum of the anthracene-picric acid charge transfer complex.

the intact radical cation at m/z 178 compared to the signal in the FAB mass spectrum of an equivalent amount of underivatized anthracene. Charge transfer derivatization reactions are useful for the FAB analysis of samples electronically but not chemically reactive, such as the polynuclear aromatic hydrocarbons. However, in general, charge transfer derivatization reactions lack the selectivity of functional group specific ion attachment derivatization reactions.

Conclusions

It has been found that charge transfer complexation reactions are useful derivatization reactions for the analysis of polynuclear aromatic hydrocarbons by FAB mass spectrometry. In most cases, the analysis should be done in the absence of a liquid matrix. Additionally, the selective analysis of biomolecules containing primary amino and hydroxyl functional groups is possible through the use of functional-group-specific ion attachment reactions.

Literature Cited

1. Bald, E.; Mazurkiewicz, B. *Chromatographia* 1980, *13*, 295.
2. DiDonato, G.C.; Busch, K.L. *Anal. Chim. Acta* 1985, *171*, 233.
3. Flurer, R.A.; Busch, K.L. In preparation.
4. Katritzky, A.R.; *Tetrahedron* 1980, *36*, 679.

