Proceedings of the Indiana Academy of Science
(1987) Volume 97 p. 165-169.

Mechanisms in Secondary Ion Mass Spectrometry: Sputtering of Preformed Ions and Reactions of Itinerant Protons

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Introduction

Prior to 1981, samples studied by mass spectrometry were limited to those compounds sufficiently volatile to vaporize without thermal decomposition. This precluded the analysis of most biologically important compounds, including steroids, peptides, and proteins. The development of "soft" ionization techniques such as fast atom bombardment (FAB) and secondary ion mass spectrometry (SIMS) have made it possible to analyze such large biomolecules.

The usual procedure of analysis in secondary ion mass spectrometry is dissolution of the sample in a suitable liquid matrix, usually glycerol, followed by bombardment with a 2-7 keV beam of energetic ions. The ions sputtered from the solution into the gas phase are mass-analyzed to produce the SIMS spectrum. One mechanistic model suggests that ionization of neutral molecules takes place in a two-step process, (i) the transfer of sample from the solution into the gas phase, and (ii) ionization of the sample molecules by various reactions, such as protonation to form $(M + H)^+$ or cationization to form, for example, $(M + Na)^+$. SIMS mass spectra usually contain a lower abundance of ions such as $(M + H)^+$ and $(M + Na)^+$ relative to intact cations of ammonium, sulfonium, idonium and other onium salts that exist in the glycerol solution as performed ions. Therefore, it is usually advantageous to convert the sample molecules to an ionic form prior to SIMS analysis. The ionization process then takes place in a single step (the sputtering of a performed ion). This single-step process can be shown to be much more efficient than the two-step process noted above. Selectivity is also enhanced if the derivatization procedure used is specific for a particular functional group in the neutral molecule. This paper describes the development of a derivatization reaction for steroids.

A study of thiamine hydrochloride has been conducted to further understand the protonation process in the selvedge, the region just above the solution/gas-phase interface (2). Even in the SIMS mass spectra of ionic compounds such as the onium salts, there is some evidence for proton transfer reactions in the creation of the fragment ions. The spectrum of thiamine hydrochloride contains ions that result from these reactions.

Methods and Materials

SIMS mass spectra were obtained on a custom-built mass spectrometer, utilizing an Extranuclear quadrupole equipped with a commercial cesium ion gun (Phrasor Scientific). The cesium ions had an energy of 6 keV, with an ion current of 1 uA.

The steroids and Girard's T reagent were obtained from Sigma Chemical Co. and used without further purification. Thiamine hydrochloride was used as received from Mallinckrodt, Inc.

Results and Discussion

Keto-steroids were derivatized with Girard's T reagent, forming a quaternary ammonium salt. The structure and SIMS mass spectrum of derivatized tetrahydrocortisone is given in Figure 1. The mass spectrum contains an abundant ion at m/z 478, corresponding to the intact cation of the derivatized molecule. This ion is well removed in mass

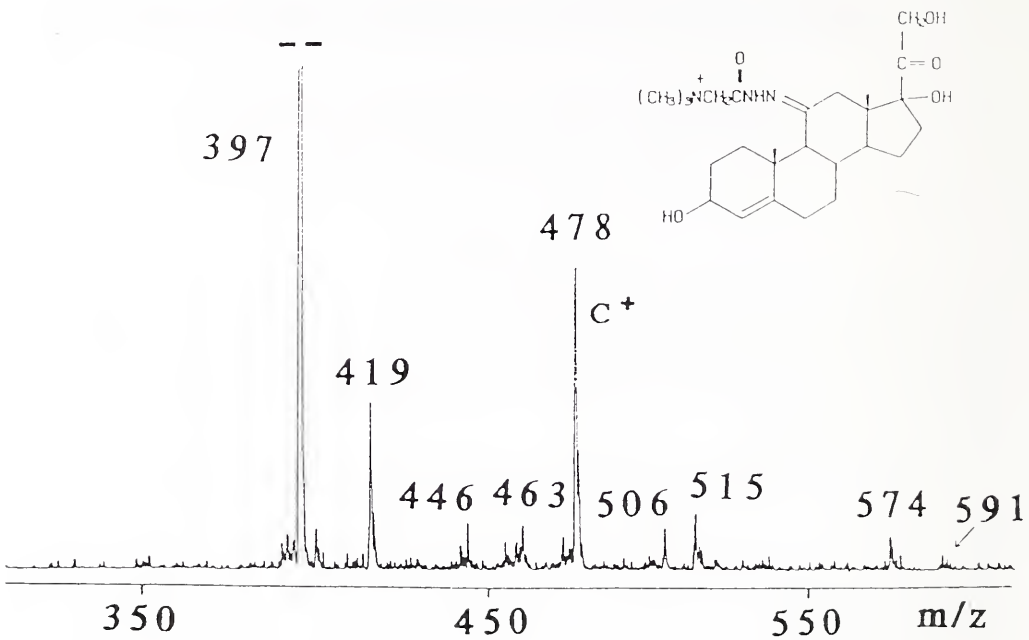

Figure 1. SIMS spectrum of tetrahydrocortisone derivatized with Girard's T reagent.

from the background ions associated with the glycerol matrix. Fragment ions are seen at m/z 463, which arises from the loss of a methyl group from the intact cation, and m/z 446, which corresponds to the subsequent loss of a hydroxy group from the ion at m/z 463. An abundant ion at 419 daltons corresponds to the loss of trimethylamine from the intact caction. This loss of trimethylamine from the intact cation is seen in the mass spectra of all steroids derivatized with Girard's T reagent.

Interestingly, ions of a higher mass than the intact cation are also observed in the spectrum. These correspond to the doubly-derivatized (note the presence of two carbonyl groups in the original structure), but singly-charged, tetrahydrocortisone ion at m/z 591, the fragment ion due to loss of a hydroxyl group from the ion at m/z 574, and an ion from the further loss of trimethylamine at m/z 515. The fact that double-derivatization can occur gives added structural information because one can deduce the number of carbonyl groups on the steroid. The reactions leading to these ions are observed in the mass spectra of all steroids investigated so far. Sensitivity is enhanced by the preformation of the ion, and structural information provided by spectral interpretation.

Thiamine hydrochloride has previously been studied by mass spectrometry using a variety of ionization techniques (1,3,4). Figure 2 shows the processes leading to the most abundant commonly observed fragment ions. The fragmentations observed with all ionization methods are similar with one important difference. Some researchers report a fragment ion at m/z 122, which is the pyrimidine fragment of thiamine, while others report a fragment at m/z 123, the protonated version of the ion at m/z 122. This difference has never been explained. Our study of thiamine hydrochloride was undertaken to provide an insight into the process through which the extra hydrogen is retained in this fragment ion.

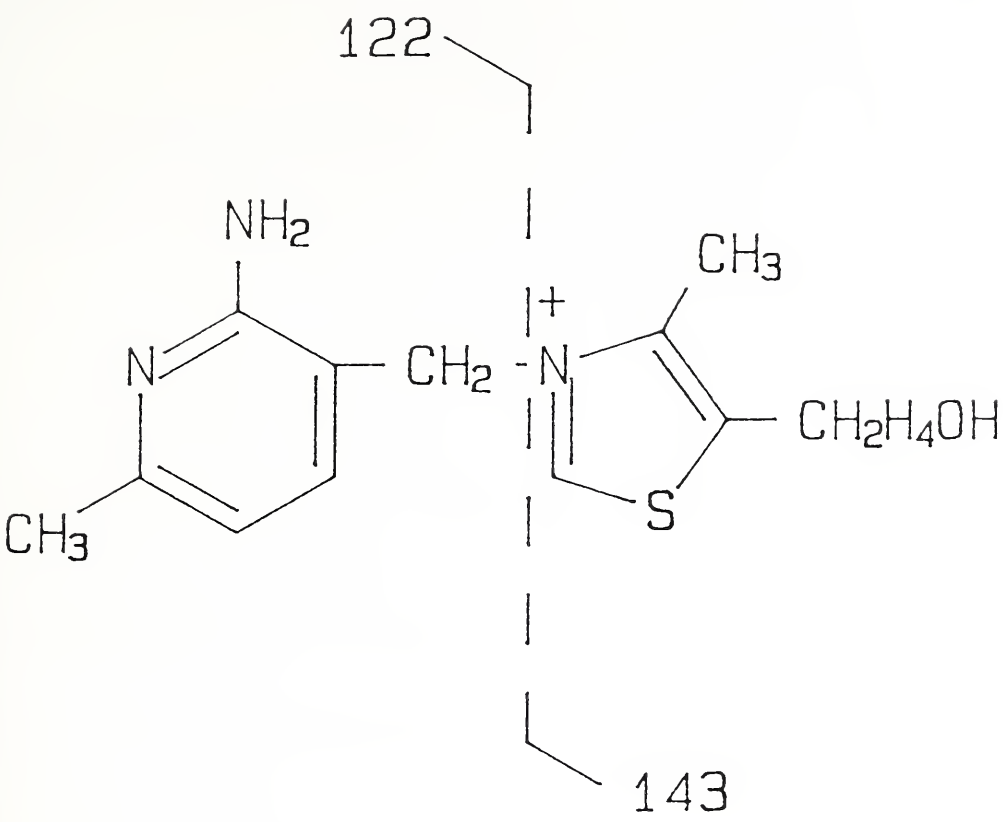

Figure 2. Fragmentation scheme of thiamine hydrochloride.

Figure 3 is the SIMS mass spectra of thiamine hydrochloride using (a) glycerol and (b) sorbitol as matrices. With the sample dissolved in glycerol, the mass spectrum contains the intact cation at m/z 265, the fragment ion at m/z 144 corresponding to the thiazolium ring, and the pyrimidine fragment ion at m/z 122. Also present is an abundant ion at m/z 123, the protonated version of the ion at m/z 122. However, when sorbitol is used as the matrix, the abundance of the ion at m/z 122 is greater than that at m/z 123.

A number of other mass spectra were recorded in which the solvent matrix was rendered more or less acidic with additives such as hydrochloric or camphorsulfonic acid. The ratio of the ions at m/z 122 and 123 were determined in each case. After many such experiments, a correlation between the abundance of these ions and the overall appearance of the spectrum was noted. We suggest that the difference in the spectra is due to a difference in composition of the selvedge region above the solution. With glycerol (Gly) as the matrix, the mass spectrum contains ions at m/z 93 $(Gly + H)^+$ and 185 $(2Gly + H)^+$, as well as those due to thiamine. However, when sorbitol is used as the matrix for dissolution and analysis of thiamine, the mass spectrum does not show any ions corresponding to sorbitol. In all cases in which the mass spectra contained solvent (S) ions $(S + H)^+$, the fragment at m/z 123 is the more abundant. If no solvent ions are seen, the ion at m/z 122 is more abundant. From this information, we can conclude that in the case of thiamine, protonation of the fragment ion occurs in the gas-phase, and the composition of the ion/solvent cluster ions in the selvedge region is the key factor. The ratio of the ions at m/z 123 and 122 is thus an indicator of the relative acidity of the selvedge created by primary ion bombardment.

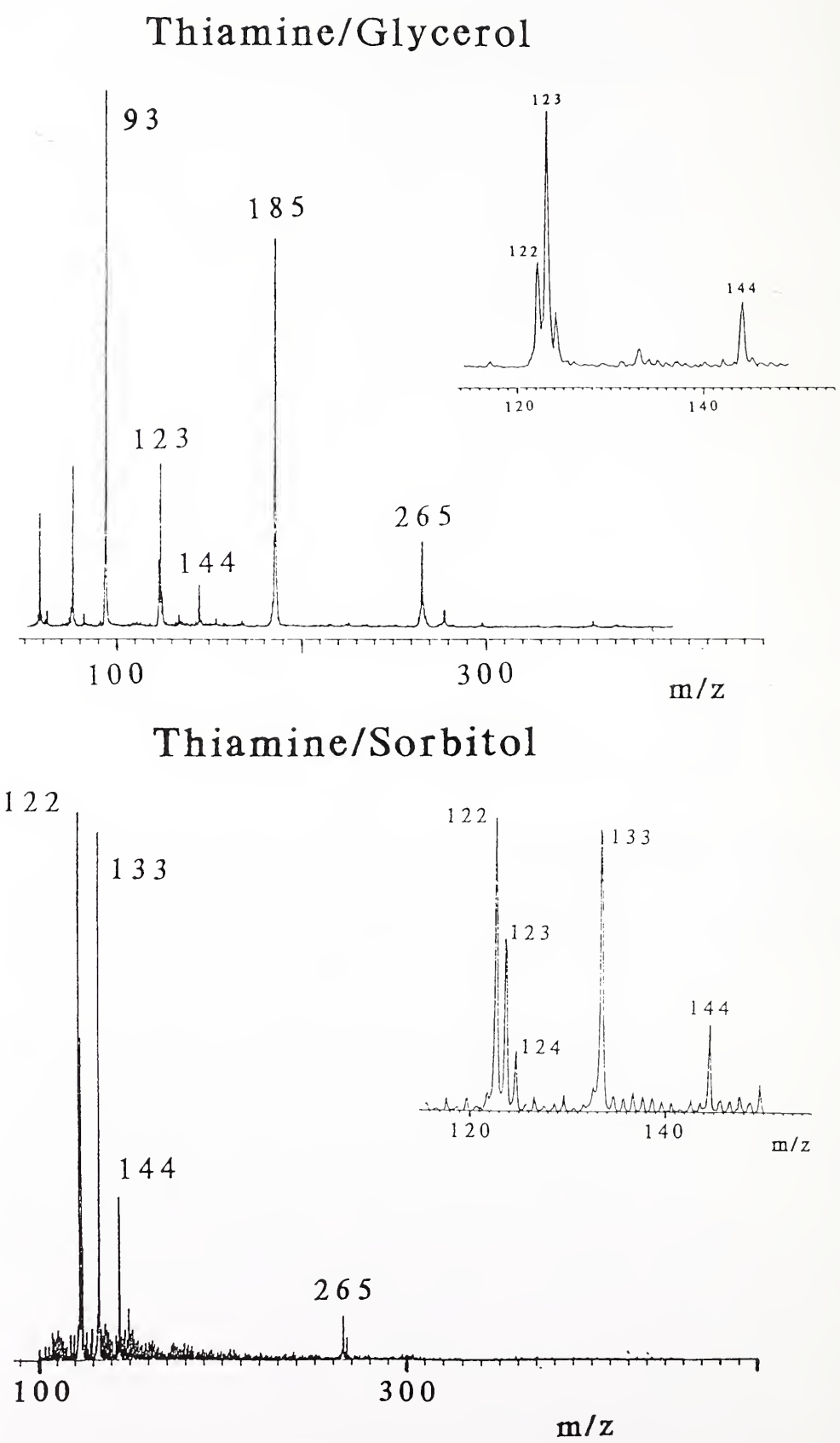

Figure 3. SIMS spectra of thiamine hydrochloride using glycerol and sorbitol as matrices.

Conclusions

It has been shown that the derivatization of keto-steroids with Girard's T reagent to form the quaternary ammonium salt greatly increases the ion yield. Also, the number of carbonyl groups in these steroids can be determined by observing multiply-derivatized species. In a more fundamental study, the protonation of the pyrimidine fragment formed from thiamine hydrochloride has been determined to be a gas-phase process.

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