Occurrence of Gas Phase Ammonolysis during Chemical Ionization Mass Spectrometry

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Ammonolysis of base-sensitive bonds such as lactone, ester, and amide bonds has been found to occur under chemical ionization mass spectrometry conditions. This reaction occurs in the mass spectra of (1) the antimycin A complex, (2) a synthetic precursor of antimycin A_3, and (3) proline dipeptides. The role that ammonolysis in CI played in the structural elucidation of the minor components of the antimycin A complex is discussed. The conditions in the ion source of the mass spectrometer ensure that a several hundredfold excess of ammonia over sample is maintained during the course of vaporizing the sample.

While investigating the antimycin A complex[1b-8] by mass spectrometry, we compared the electron ionization (EI) and chemical ionization (CI) mass spectra of this complex. Instead of the typically "clean" CI spectrum that one normally obtains when ammonia is used as a reagent gas[9-12] (proton affinity of NH_3 207 ± 3 kcal/mol[13]), as many peaks were found in the CI spectrum with ammonia as the reagent gas as in the EI spectrum. This can only be rationalized by the occurrence of gas phase ammonolysis: new compounds (amides) are formed in the ion source which then subsequently are protonated by the ammonia ion-molecule reaction products[12] to produce protonated molecular ions. Accordingly, we investigated the occurrence of gas phase ammonolysis in the CI spectra of (1) the antimycin A complex, (2) a synthetic precurser of antimycin A_3[14,15] which does not contain any homologs, and (3) a series of proline dipeptides.

The observance of gas phase ammonolysis products was especially useful in the case of the antimycin A complex. This complex is composed of a mixture of homologous compounds, due to the presence of two alkyl side chains (structure I). While investigating the EI spectra of this complex, we found that each molecular species consists of one to four components.[16] These constituents are extremely difficult to separate.[5,6] With methane as a reagent gas for CI, adduct ions were obtained which differed by 28 amu (just as every other homolog did), whereas ammonia helped to establish the molecular ions, as each molecular species is indicated by $[M + 1]^+$ and $[M + 18]^+$ ions.[12]

Results and Discussion

The Antimycin A Complex. Figure 1a contains the EI mass spectrum of the antimycin A complex (I). Scheme I rationalizes the genesis of the indicated ions. These data have also been confirmed by the mass spectra of II–V.

Figure 1b contains the CI spectrum of I. By comparing the two mass spectra it is apparent that all of the ions in the molecular ion region of the EI spectrum have shifted 1 and 18 amu in the CI spectrum. This shift provides sufficient information to determine which molecular components are present in the complex by the analysis of the $[M + H]^+$ and $[M + NH_4]^+$ ions shown in Table I. The quantity $(R_3 + R_4)$ is a convenient representation of the sum of the number of carbon atoms present in the two aliphatic side chains, R_3 and R_4, as shown in Scheme II.

The "shift" of fragment ions by either 17 or 18 amu is the indication that ammonolysis is occurring. The intact molecule (I) contains five potential sites where ammonolysis of a base-sensitive bond may occur—two lactone, one ester, and two amide bonds. For example, the amide bond next to the nine-membered ring is one of the potential sites. If ammonolysis takes place, the ion found at m/e 164 would "shift" 17 amu (owing to the addition of an $-NH_2$ moiety followed by protonation) to form the molecular ion u at m/e 181. In Figure 1b, we observe the appropriate shift.

Table I

No. of carbons $(R_3 + R_4)$	m/e $(M + 1)^+$	m/e $(M + 18)^+$
5	479	
6	493	510
7	507	524
8	521	538
9	535	552
10	549	566
11	563	580
12	577	594

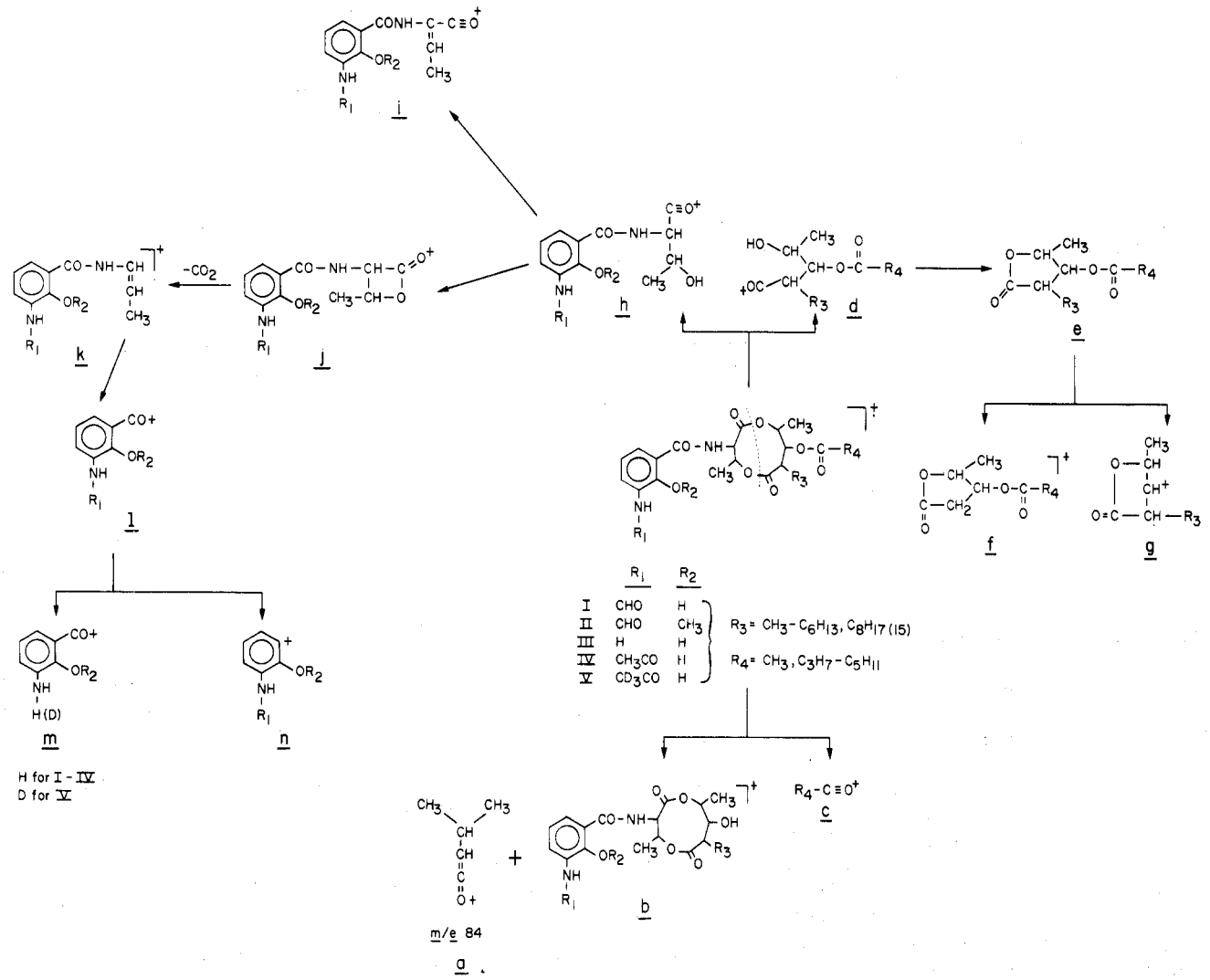
An interesting reaction occurs with the asymmetrically disubstituted dilactone ring of antimycin A. The two extremely labile lactone bonds both undergo ammonolysis to form two hydroxy amides, which after protonation form the molecular ions r and s (see labeled peaks in Figure 1b). In order to obtain corroborative evidence that ammonolysis is occurring, the CI spectrum was also run with ND_3 as the reagent gas, whereupon the appropriate shift in mass occurred. (For example, m/e 274 → 278 is a shift of 4 amu and is composed of two deuteriums on the $-ND_2$ group from the reagent gas, the deuterium on the -OD group, and a deuterium to deuterate the molecule.)

The other moiety formed after ammonolysis of the dilactone ring and protonation is the molecular ion s, m/e 282, which readily loses NH_3 to form the more stable lactone t at m/e 265.

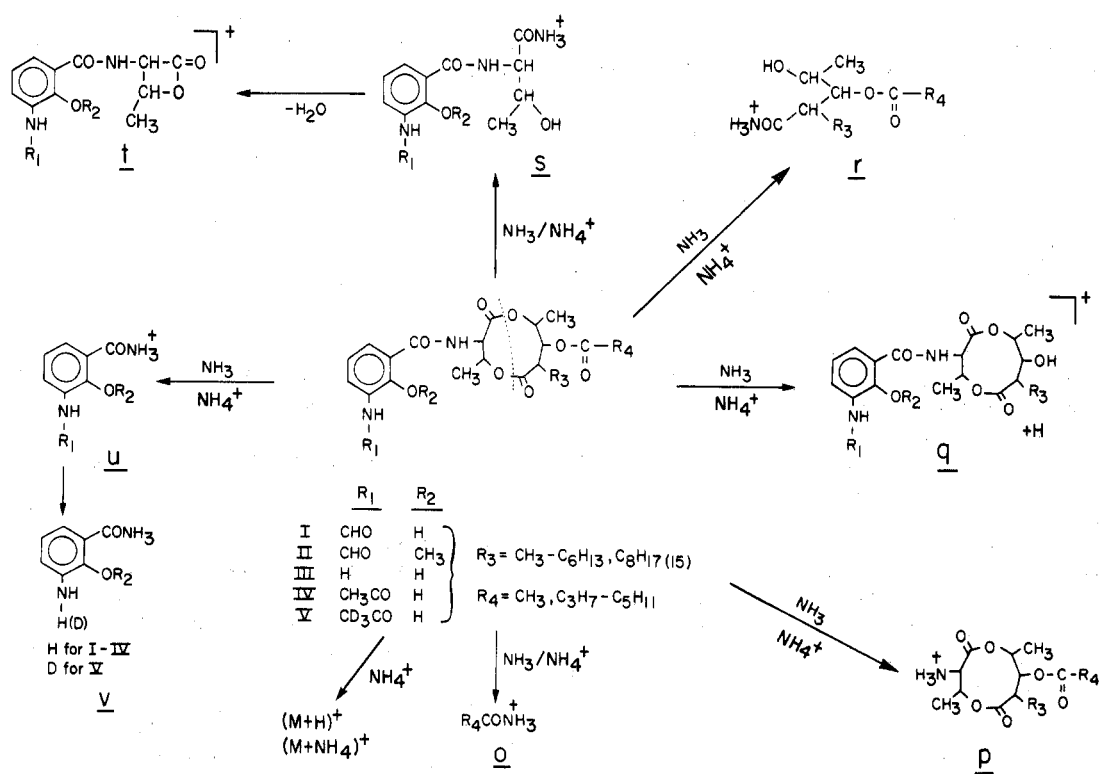
The ions which are found in Figure 1b do not represent extensive fragmentation of the molecule, but rather, every peak in this mass spectrum represents a molecular ion. That some of these ions are still low in abundance is due to their low concentration in the antimycin A complex.[1b] Generally the abundance of these ions is much greater in the CI spectrum (as compared to the corresponding ions in the EI spectrum) because they do represent nonfragmented molecular ions. With ammonia as a reagent gas, the sensitivity toward nitrogen-containing compounds (or conjugated compounds) is higher in that these compounds represent stronger Brønsted acids than the reactant ion NH_4^+.

Ammonolysis can be shown to occur in a base-labile compound that cleaves into two moieties of different molecular weights followed by the detection of both species. A model compound containing no homologs was reported during the total synthesis of antimycin A_3 and its diastereoisomer.[14,15] This compound is the benzoxycarbonyl derivative of the dilactone ring in structure I and is (−)-(3*S*,4*R*,7*S*,8*S*,9*R*)-3-benzyloxycarboxamido-7-butyl-4,9-di-

Scheme I



Scheme II



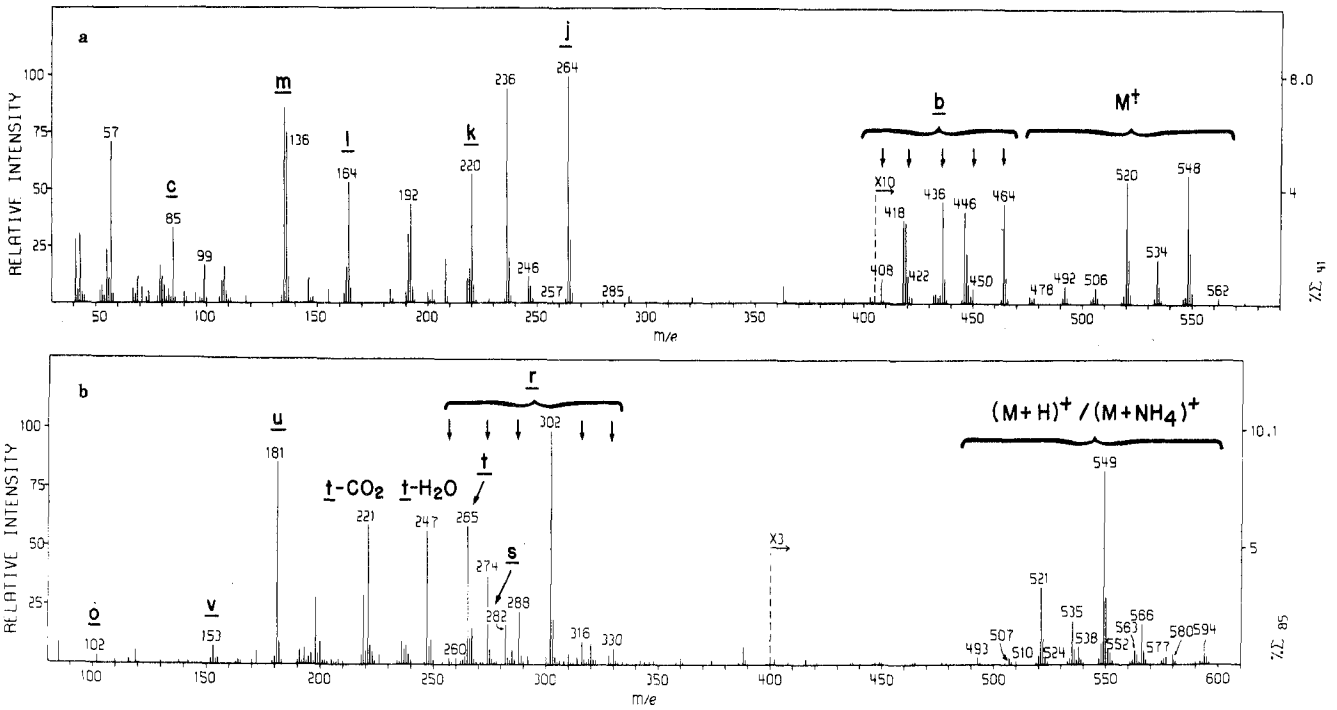


Figure 1. (a) EI mass spectrum of antimycin A complex (I); (b) CI (NH_3) mass spectrum of antimycin A complex (I).

methyl-1,5-dioxa-8-isovaleryloxycyclononane-2,6-dione (VI, Scheme III).

The *m/e* values and the relative intensities from the CI mass spectrum of VI with ND_3 as the reagent gas are given in Scheme III. The molecular weight (M) of VI is 491 amu. With ND_3 as the reagent gas, exchange of the amide hydrogen occurs quantitatively. Deuteration then produces the ion at *m/e* 494 (5%), while addition of ND_4^+ produces the base peak at *m/e* 514. Opening of the dilactone ring only at one point and subsequent deuteration yields the two molecular ions w and x at *m/e* 514, coincident in mass with the $[M + ND_4]^+$ ions (see Scheme III).

Ammonolysis of the amide bond in VI followed by deuteration produces ion y at *m/e* 361 (20%). The other product of this reaction is the unstable carbamic acid ester (ion z), which is not found.

The base-sensitive dilactone ring of VI undergoes ammonolysis to generate, after deuteration, ions aa (80%) and ab (15%) at *m/e* 278 and 258, respectively. (Some of the ion current at *m/e* 278 may be due to the addition of ND_4^+ to ab.)

Ammonolysis of the aliphatic ester bond of VI does not seem to occur owing to the absence of ions ac and ad. This could be explained by the greater stability of the aliphatic ester bond in comparison to the more base-labile dilactone ring.

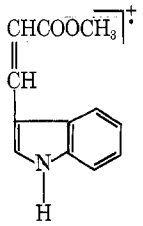
It is interesting to make some general observations concerning the spectra in Figures 1a, 1b, and 2 and Scheme III. For example, the more abundant ion species in the EI spectrum (Figure 1a) are due to the aromatic portion containing ions, such as those occurring at *m/e* 264, 220, 164, and 136, whereas the aliphatic ions (Scheme I, d, e, f, and g) are quite small. On the other hand, the situation is quite different in the CI spectrum (Figure 1b). For example, r in Scheme II accounts for the family of abundant ions at *m/e* 260, 274, 288, 302, 316, and 330 in Figure 1b.

The characteristic abundant ions of the EI spectra reflect the presence of the aromatic portion in the antimycin A complex. An ion structure such as j (Scheme I) could quite easily be ionized by the removal of an electron.

In CI, however, with NH_3 as the reagent gas, protonation can occur only with a molecule having a higher proton affinity than NH_3. This means that the corresponding molecule to ion r has a higher proton affinity than NH_3, and therefore is more abundant (see Figure 1b) in CI as opposed to EI (Figure 1a).

Proline Dipeptides. The CI mass spectra (with NH_3) of the following eight dipeptides N^{α}-acetyl-Pro-X-methyl ester, where X = glycyl, alanyl, seryl (-OMe), leucyl, methionyl, phenylalanyl, tyrosyl (-OMe), and tryptophyl residues, showed that ammonolysis of the peptide bond occurs, too. For purposes of illustration, the Pro-Trp dipeptide will serve as an example.

The EI mass spectrum of *N*-acetylprolyltryptophanmethyl ester (VIII) is given in Figure 2a. The molecular ion occurs at *m/e* 357 and $[M - 31]^+$ at *m/e* 326. The large ion at *m/e* 201 occurs *via* a McLafferty rearrangement of the β hydrogen on the Trp side chain resulting in ion ae.



ae, *m/e* 201

The other ions are represented in the fragmentation pattern given in Figure 2a. The small ion at *m/e* 157 is due to cleavage of the N^{α}-C^{α} bond at the Trp residue, with migration of two hydrogen atoms, which then would eliminate ammonia to produce the amino acyl fragment at *m/e* 140.[16]

In each one of the CI mass spectra of the dipeptides with NH_3 as the reagent gas, the ion at *m/e* 157 was dominant. As cleavage of the N^{α}-C^{α} bond is energetically unfavorable under CI conditions with ammonia as the reagent gas, ammonolysis of the peptide bond occurred to produce ions af and ag.

The CI mass spectrum of VII, with ND_3 as the reagent gas, is presented in Figure 2b. The $[M + D]^+$ ion is observed at *m/e* 361. The molecular weight is 357 amu.

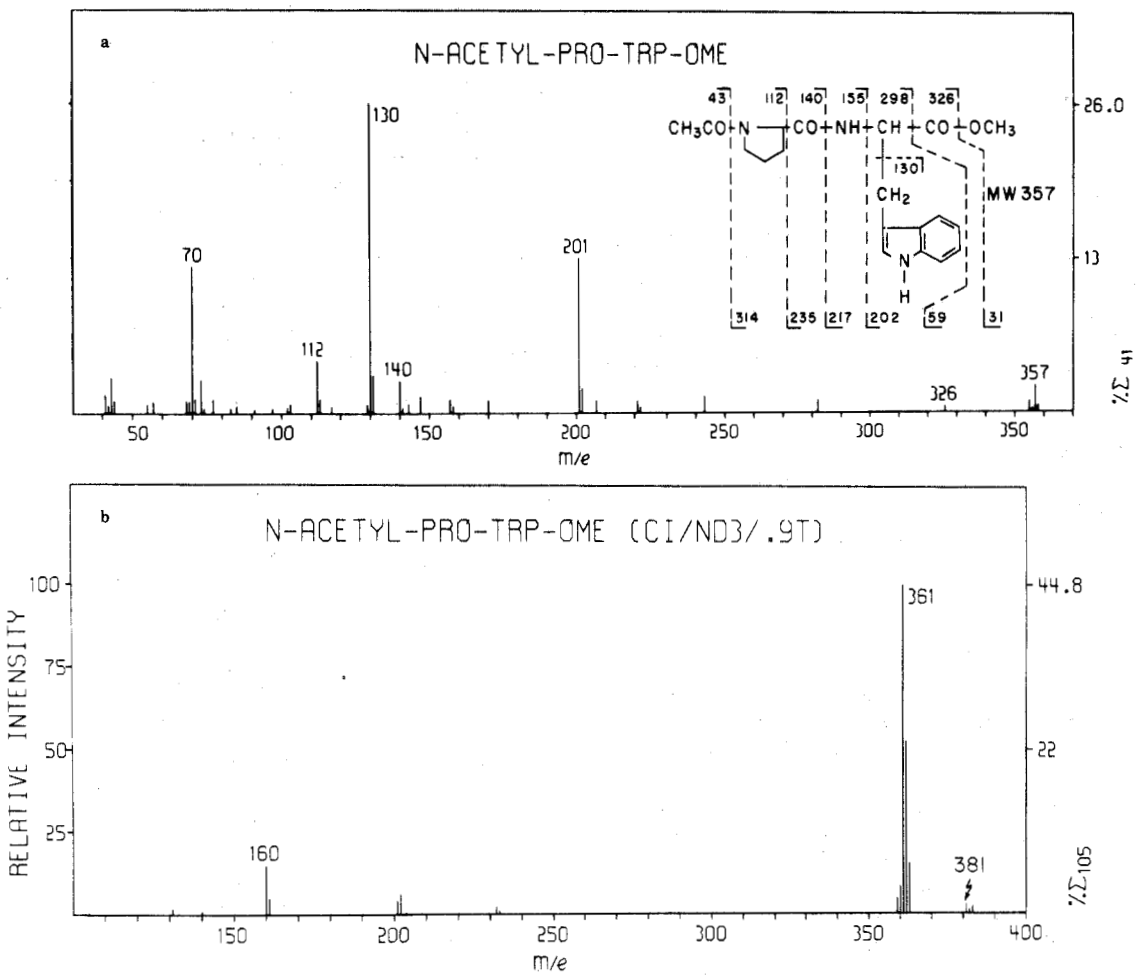
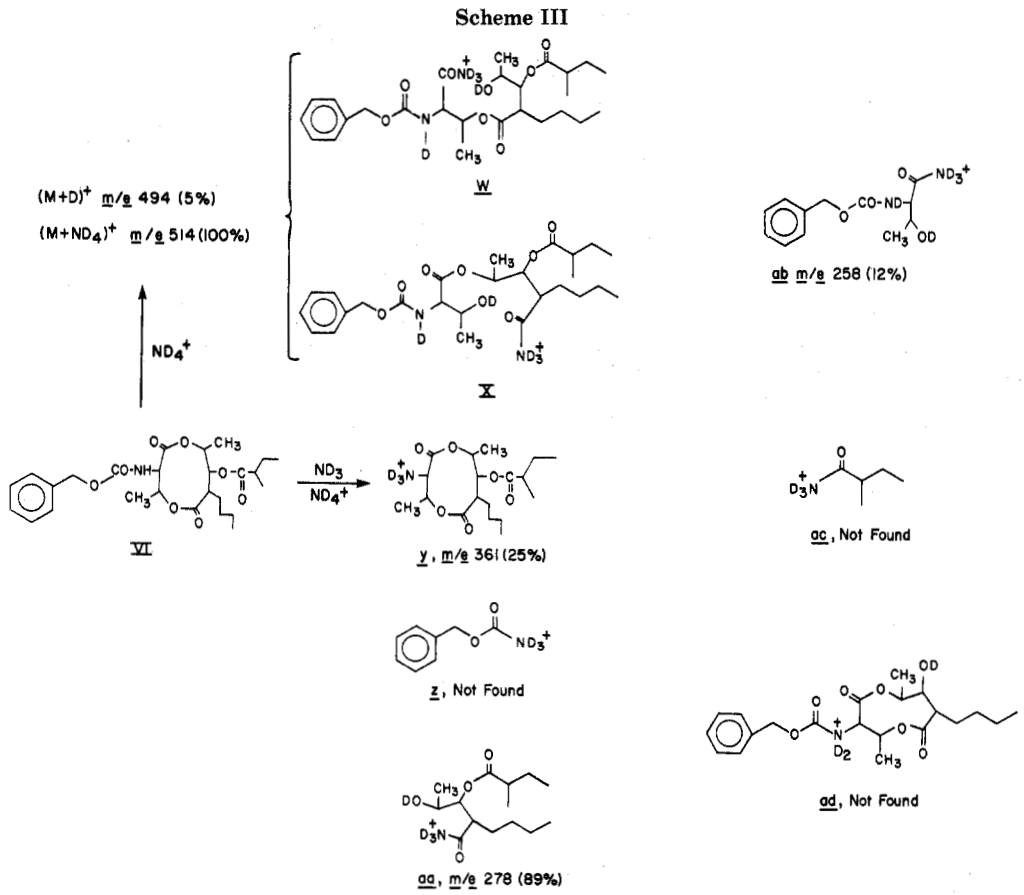
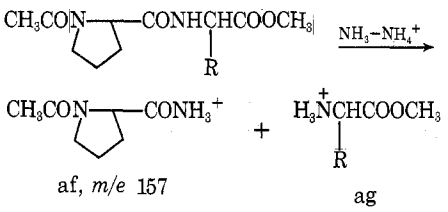


Figure 2. (a) EI mass spectrum of N-acetylated prolyltryptophan methyl ester (VII); (b) CI (ND_3) mass spectrum of N-acetylated prolyltryptophan methyl ester (VII).

Scheme III

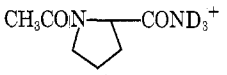




af, *m/e* 157 ag

(Two hydrogens exchange for deuterium atoms, most probably the hydrogens on the indole nitrogen and on the amide bond nitrogen. This exchange is followed by deuteration.) The adduct ion $[M + ND_4]^+$ occurs at *m/e* 381. The McLafferty rearrangement ion (ae) remains at *m/e* 201, with the satellite peak *m/e* 202 caused by deuterium scrambling of the indole hydrogen or by deamination of ag.

Under the low-energy conditions of CI, the ion at *m/e* 160 is persistently found as an abundant ion in all eight dipeptide spectra.



ah, *m/e* 160

Conclusions

The data presented above show that ammonolysis of a variety of chemical bonds occurs under CI mass spectrometry conditions. The chemical bonds affected are amide, ester, and lactone bonds. As these types of bonds are base sensitive in solution chemistry, it is not surprising that ammonolysis in the gas phase occurs. Indeed, in solution chemistry, dipeptide amides are synthesized by subjecting diketopiperazines to ammonolysis.[17]

A sufficient molar excess of NH_3 in the ion source of the mass spectrometer exists to ensure that reaction would proceed to the formation of the ammonolysis products. At a pressure of 1 Torr NH_3 in the source at 500°K, 50 μmol of NH_3 are present. By means of a heated direct introduction probe, a total amount of 10 μmol of the sample is introduced into the ion source over a period of 100–300 sec. Under normal operating conditions, a continual molar excess of several hundredfold of NH_3 over sample is assured.

Ammonolysis of base-sensitive bonds (a reaction which is standard in solution chemistry) has never been reported before to occur under the conditions of CI mass spectrometry. The conditions employed in this study are typical for CI mass spectrometry and have been utilized by others to study other classes of compounds.[10,11]

Experimental Section

The proline dipeptides were purchased from Fox Chemical Co., Los Angeles, Calif., or Sigma Chemical Co., St. Louis, Mo. They were acetylated in methanol–acetic anhydride (4:1) for 4 hr.[18] The esterification was completed by reaction with diazomethane.

The antimycin A complex was purchased from Nutritional Biochemicals Co., Cleveland, Ohio, and acetylated and methylated as above. Deformylation was done by treating a methanolic solution with concentrated HCl.[2] Compound VI was kindly provided by Dr. Mitsuhiro Kinoshita, Keio University, Yokahama, Japan.

CI mass spectra were obtained with a CEC 21-110 B (lot 9) instrument, modified for high-pressure work.[19] Ammonia and methane pressures were 0.1–0.9 Torr, as measured with a hollow probe connected to a capacitance manometer (MKS Instruments, Inc., Burlington, Mass.). Instrumental conditions follow: ionizing voltage, 200 eV; ionizing current, 200 μA (total, unregulated); accelerating voltage, −8.4 kV; magnetic scanning. The repeller voltage was set at 0 V/cm. Source temperature varied from 105 to 225°, depending upon the temperature necessary to vaporize each individual sample.

Ammonia and methane were of research grade and were purchased from Matheson LaPorte, Tex.

Low-resolution (EI) mass spectra were obtained on an LKB 9000. Samples were introduced on a direct introduction probe (ionizing voltage, 70 eV; ionizing current, 60 μA; accelerating voltage, −3.5 kV; source temperature 270°; probe temperature, 130–140°).

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