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Ammonia Chemical Ionization Tandem Mass Spectrometry in Structure Determination of Alkaloids. I. Pyrrolidines, Piperidines, Decahydroquinolines, Pyrrolizidines, Indolizidines, Quinolizidines and an Azabicyclo[5.3.0]decane

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CI-MS/MS of alkaloids with ammonia reagent gas and collision-induced dissociation provides unique structural information for certain mono- and bi-cyclic alkaloids. This technique was applied to solve the structures of *195C*, a 4,6-disubstituted quinolizidine alkaloid found in frog skin and an ant, and *275A*, a novel 3,5-disubstituted azabicyclo[5.3.0]decane found in frog skin. Copyright © 1999 John Wiley & Sons, Ltd.

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Mass spectrometry in the electron-impact mode (EI-MS) is a classical technique used to provide molecular structure information. It has excellent sensitivity but there are limitations in that it rarely provides for a complete structural determination. It has been used extensively to provide important structural information on relatively simple monocyclic and bicyclic alkaloids obtained from frog skin.[1] In EI-MS, an energy-rich molecular radical-ion ($M^{+\cdot}$) is formed that fragments by ejecting a radical to yield an even-electron daughter ion. Fragments from cleavage at bonds α to nitrogen often dominate spectra. The daughter ions may cleave further, usually yielding other even-electron ions.

In the chemical ionization mode (CI-MS), with ammonia gas as the proton donor, the protonated molecular ion of alkaloids is efficiently detected, because alkaloids are easily protonated even with a mild reagent like ammonia. Non-basic impurities in alkaloid samples, having lower proton affinities, remain mainly unprotonated and often are not detected.[2]

Tandem mass spectrometry (MS/MS) with EI ionization techniques can be a complementary and efficient alternative to regular EI-MS, because it provides detailed insights into the pathways of fragmentation that are not revealed by normal EI-MS. In addition, EI-MS/MS can be very useful for the analysis of compounds present in complex mixtures. Thus, coeluting compounds, often a problem with liquid or gas chromatography of complex mixtures, does not prevent a detailed analysis of parent and daughter ions of each of the coelutants by MS/MS.[3,4] However, in the case of coeluting stereoisomers with parent ions of the same mass, such analysis by EI-MS/MS is compromised.

In tandem mass spectrometry of alkaloids in the CI mode, the first step is protonation of the N-atom in the alkaloid providing $[M+H]^+$, an even-electron ion usually of lower internal energy than the radical molecular ion formed under EI conditions. Since the $[M+H]^+$ ion is relatively stable, there is little fragmentation in the normal CI mode, and the mass spectrum consists mainly of the protonated parent molecule. In order to induce fragmentation of $[M+H]^+$, it is necessary to increase its energy, usually by allowing collision with a collision gas to produce a collision-induced dissociation (CID). A second mass spectrum is then obtained from the activated protonated parent molecule. The most important feature with respect to fragmentation of alkaloids is that the $[M+H]^+$ ion cannot release a radical, because the valence of the N-atom is saturated and therefore the fragmentation pathways are quite different from those of EI-MS/MS.

There have been several studies on CI-MS/MS with respect to fragmentation pathways of organic compounds. Fragmentations of naturally occurring morphinan isomers were studied after protonation with ammonia or methane, and mechanistic analysis of stereospecific decomposition pathways were performed and corroborated using deuterium labeling.[5] Phthalic acid and related compounds were studied by positive- and negative-ion CI-MS/MS and the principal fragmentation pathways established.[6] CI-MS/MS has been employed in the structural determination of complex biomolecules such as glycoconjugates.[7]

We now provide a systematic study of CI-MS/MS as applied to the following classes of simple monocyclic and bicyclic alkaloids: Pyrrolidines, piperidines, decahydro-

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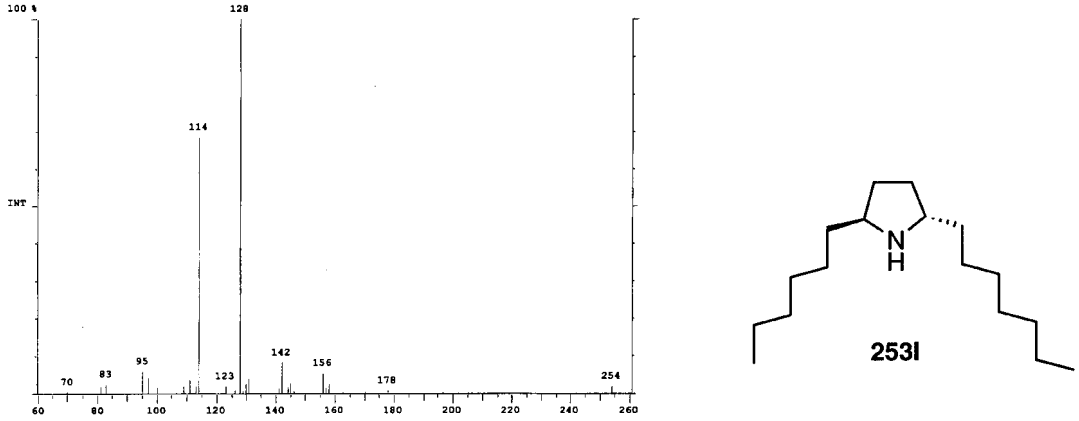


Figure 1. CI-MS/MS spectrum of **235I**, *trans*-2-*n*-heptyl-5-*n*-hexylpyrrolidine.

quinolines, and a group of 'izidine' alkaloids, including pyrrolizidines (two fused five-membered rings), indolizidines (fused six- and five-membered rings), quinolizidines (two fused six-membered rings), and azabicyclo[5.3.0]decanes (fused seven- and five-membered rings). All of the alkaloids in the present study are disubstituted. Most of these disubstituted alkaloids occur in complex mixtures isolated from extracts of amphibian skin.[1] Some also occur in ants.[8] While some of these alkaloids are synthetic, all of them represent structures or substructures widely distributed among natural products and, thus, such CI-MS/MS data will be useful in the structural determination of natural products, where the availability of material is scarce.

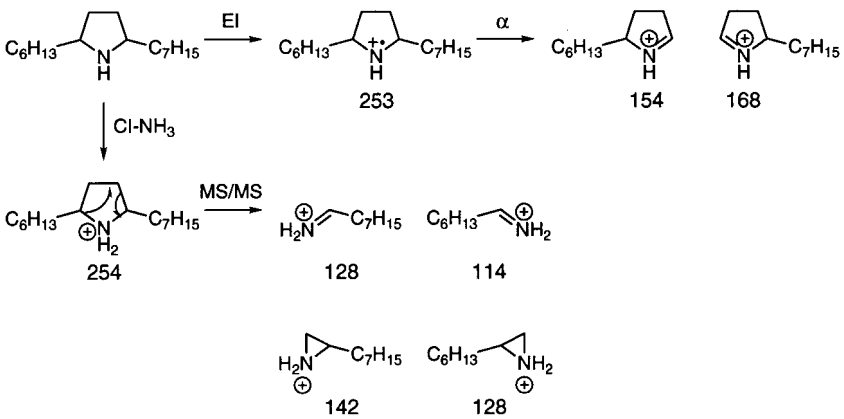
EXPERIMENTAL

A Finnigan (San Jose, CA, USA) GCQ mass spectrometer and gas chromatograph fitted with an RTX-5MS (Restek) column (30 m × 0.25 mm i.d.) programmed from 150 to 280 °C at 10 °C /min was used with NH_3 in the CI mode to generate CI-MS/MS spectra of all alkaloids, and in the EI mode to generate EI and, in some cases, EI-MS/MS spectra. Helium carrier gas was used as collision gas.

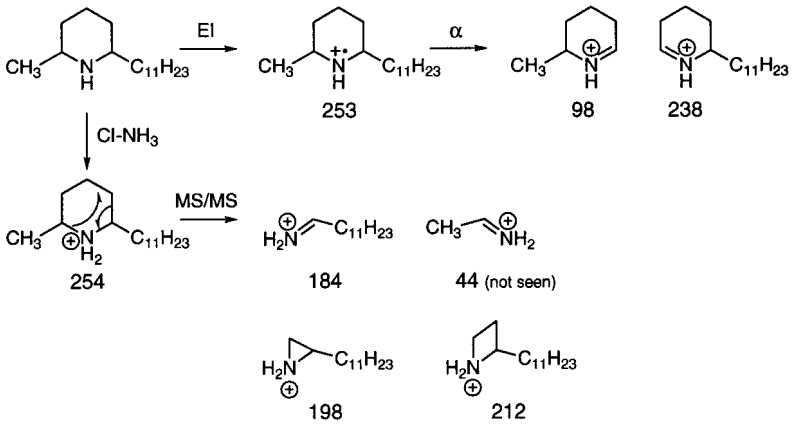
Alkaloids **cis-195A, cis-219A, 223AB, 205A, 207A, 207A″, 209B, 195C** and **275A** were present in extracts from frog skin as reported.[1] Alkaloids **253I** and **253J** were present in extracts from ants as reported.[8] The synthetic alkaloid (−)-1*R*,4*S*,10*S*-4-allyl-1-ethylquinolizidine[9] was provided by T. Momose (Toyama Medical and Pharmaceutical University, Toyama, Japan). Perhydro-**275A** and (1*R*,4*S*,10*S*)-1-ethyl-4-propylquinolizidine were obtained by hydrogenation in methanol (H_2–Pd/Al_2O_3) of an extract containing alkaloid **275A** and of a sample of (−)-1*R*,4*S*,10*S*-4-allyl-1-ethylquinolizidine, respectively.

RESULTS AND DISCUSSION

Comparison of the mass spectra of relatively simple monocyclic and bicyclic alkaloids of several structural classes reveals the markedly different fragmentation pathways for the molecular radical ion in the EI mode versus fragmentation pathways for the protonated molecular ion in the CI mode after activation by CID. A common feature for EI-MS in the relatively simple monocyclic and bicyclic alkaloids of the present study is the loss of a substituent as a radical from the position adjacent (α) to the N-atom. The most important fragment ions in EI-MS for the various mono- and bicyclic alkaloids are shown in Schemes 1 to 9 for comparison with the fragmentations seen after CI-MS/MS. The EI mass spectra documented here were obtained with the same ion trap instrument that was used to obtain the CI-MS/MS spectra. Such spectra can be called 'ion trap EI mass spectra', since they can differ slightly from spectra obtained under true EI conditions in a magnetic sector instrument, because of the much longer flight times ions experience in an ion trap. A common feature for CI-MS/MS



Scheme 1. Proposed EI and CI-MS/MS fragmentation pathways for a 2-*n*-heptyl-5-*n*-hexylpyrrolidine.



Scheme 2. Proposed EI and CI-MS/MS fragmentation pathways for a 2-methyl-6-*n*-undecylpiperidine.

in the present series of alkaloids is cleavage with rearrangements, since radical fragmentation is less likely to occur. CI-MS/MS fragmentation pathways for the various mono- and bicyclic alkaloids are also proposed in Schemes 1 to 9.

2,5-Disubstituted pyrrolidines

The pyrrolidines may fragment on NH_3-CI-MS/MS as proposed in Scheme 1 for 2-heptyl-5-hexylpyrrolidine. The spectrum is shown in Fig. 1. The major diagnostic fragments are two even-mass ions, in this case at *m/z* 114 and 128. Two other even-mass ions might be expected, since a similar mechanism would yield the ions with a three-membered ring, in this case at *m/z* 128 and 142. A low intensity ion *is* present at *m/z* 142 and consequently it is likely that the peak at *m/z* 128 consists of two species. Terminal double bonds have little effect except that a new peak at $[M+H-42]^+$ is seen due to loss of propene (data not shown). When two terminal double bonds are present, a second loss of 42 Da. is observed, yielding $[M+H-84]^+$ (data not shown). *cis*- and *trans*-Disubstituted pyrrolidines yield very similar spectra (data not shown).

2,6-Disubstituted piperidines

It is proposed that the piperidines fragment on NH_3-CI-MS/MS in a manner similar to the pyrrolidines, as shown in Scheme 2 for 2-methyl-6-undecylpiperidine. The spectrum is shown in Fig. 2. In this case, one of the substituents is a methyl group, but the expected ion at *m/z* 44 is not present, probably because the other fragmentation, in this case to *m/z* 184, is strongly favored. Equivalent ions with a three- and a four-membered ring are also observed, in this case at *m/z* 198 and 212, respectively. Elimination of one or two propene units occurs when the disubstituted piperidine has one or two terminal double bonds, respectively (data not shown). Odd-mass ions, in some cases very intense peaks, are probably hydrocarbon ions, formed after an initial heterolytic cleavage that results in loss of the N-atom in a neutral fragment such as NH_3. *cis*- and *trans*-Disubstituted piperidines yield very similar spectra (data not shown).

2,5-Disubstituted decahydroquinolines

The decahydroquinolines contain a piperidine ring, but the NH_3-CI-MS/MS fragmentation is remarkably different from that of the piperidines (see above), yielding virtually only odd-mass, presumably hydrocarbon peaks at *m/z* 81, 95, 109, etc. (Scheme 3). The spectrum of *cis*-**195A** (*cis*-5-methyl-2-propyldecahydroquinoline) is shown in Fig. 3(a). Following the loss of NH_3, the resulting hydrocarbon peak may suffer facile cleavage to smaller hydrocarbon peaks. Such odd-mass peaks (*m/z* 81, 95, 109) are also present in pyrrolidines and piperidines with an olefinic side chain (data not shown). When the side chains are saturated in these

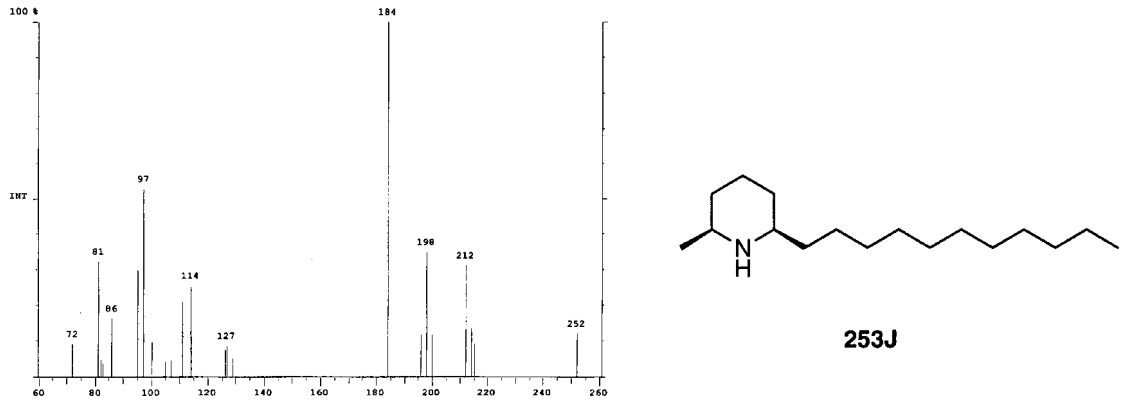
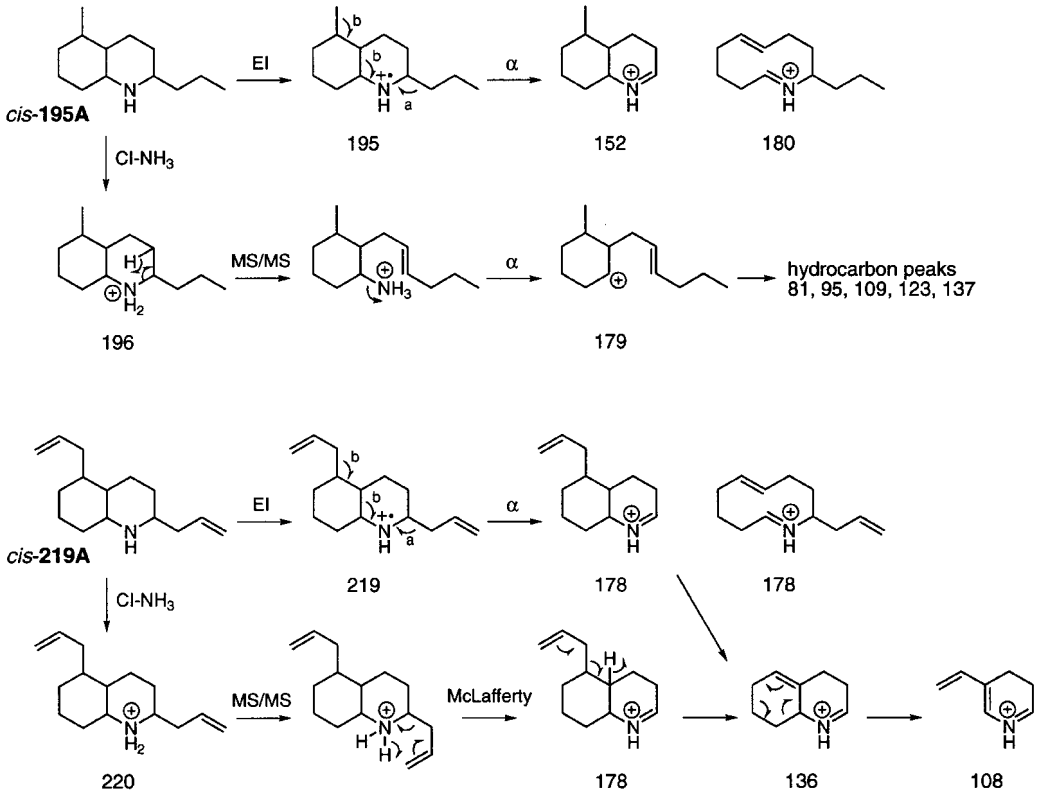


Figure 2. CI-MS/MS spectrum of **235J**, *cis*-2-methyl-6-*n*-undecylpiperidine.



Scheme 3. Proposed EI and CI-MS/MS fragmentation pathways for two *cis*-fused 2,5-disubstituted decahydroquinolines, *cis*-**195A** and *cis*-**219A**.

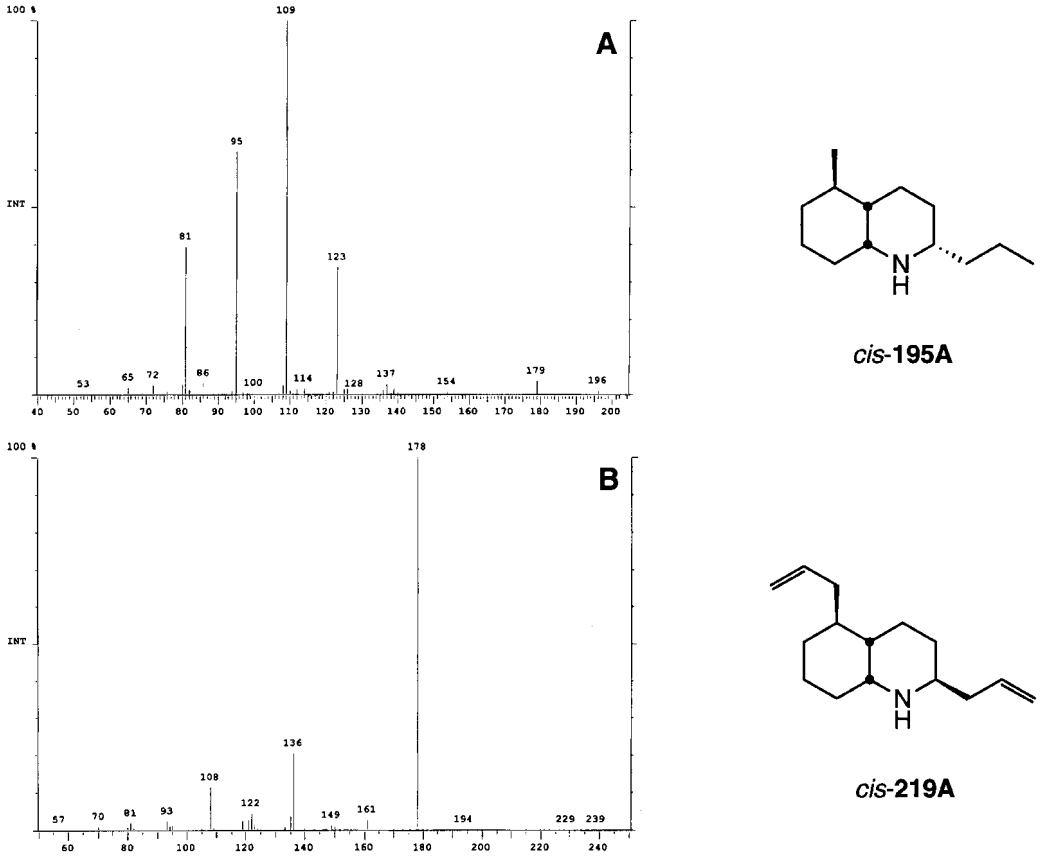
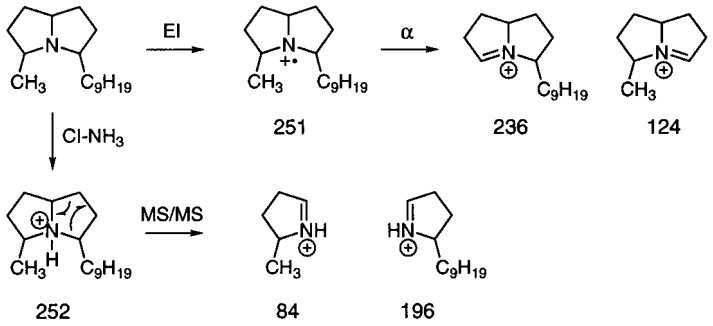


Figure 3. CI-MS/MS spectra of *cis*-fused decahydroquinolines, *cis*-**195A** (a) and *cis*-**219A** (b).



Scheme 4. Proposed EI and CI-MS/MS fragmentation pathways for a 3-methyl-5-*n*-nonylpyrrolizidine.

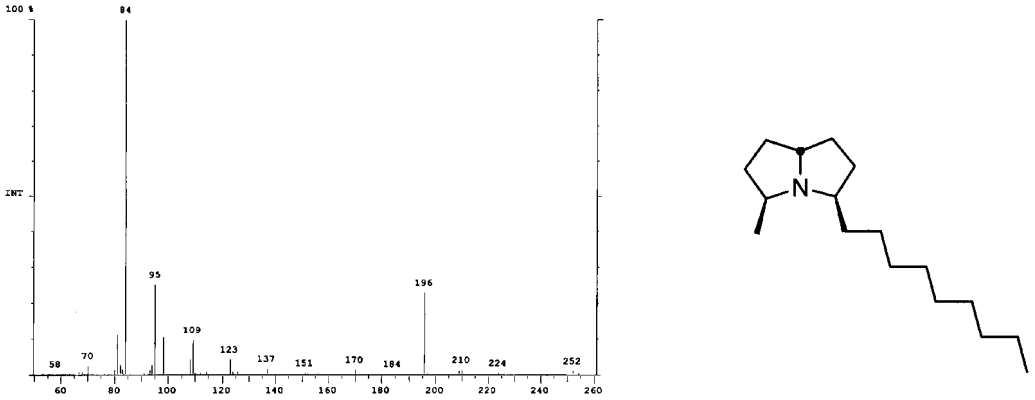
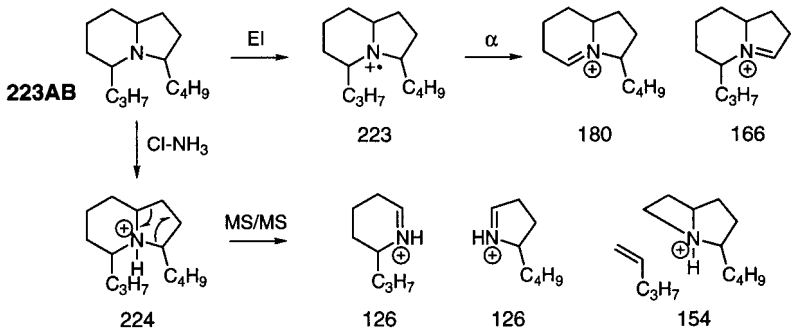


Figure 4. CI-MS/MS spectrum of (5*Z*,8*E*)-3-methyl-5-*n*-nonylpyrrolizidine.



Scheme 5. Proposed EI and CI-MS/MS fragmentation pathways for a 3-*n*-butyl-5-*n*-propylindolizidine.

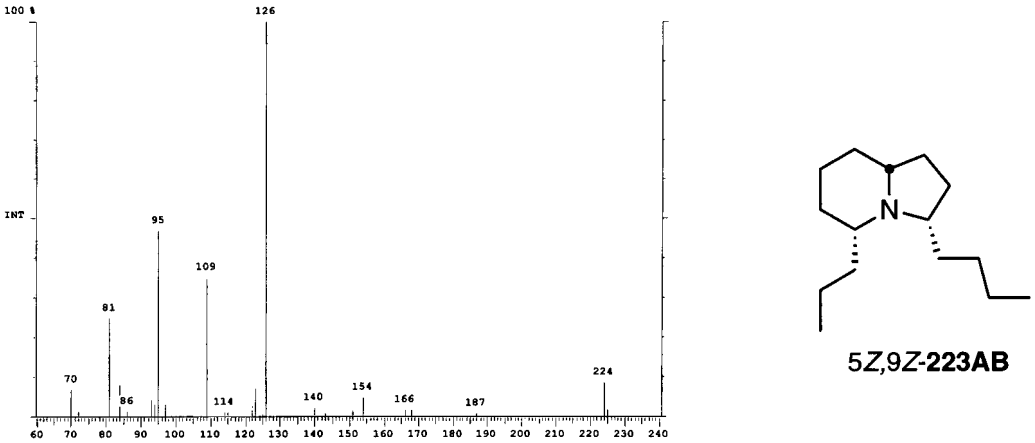
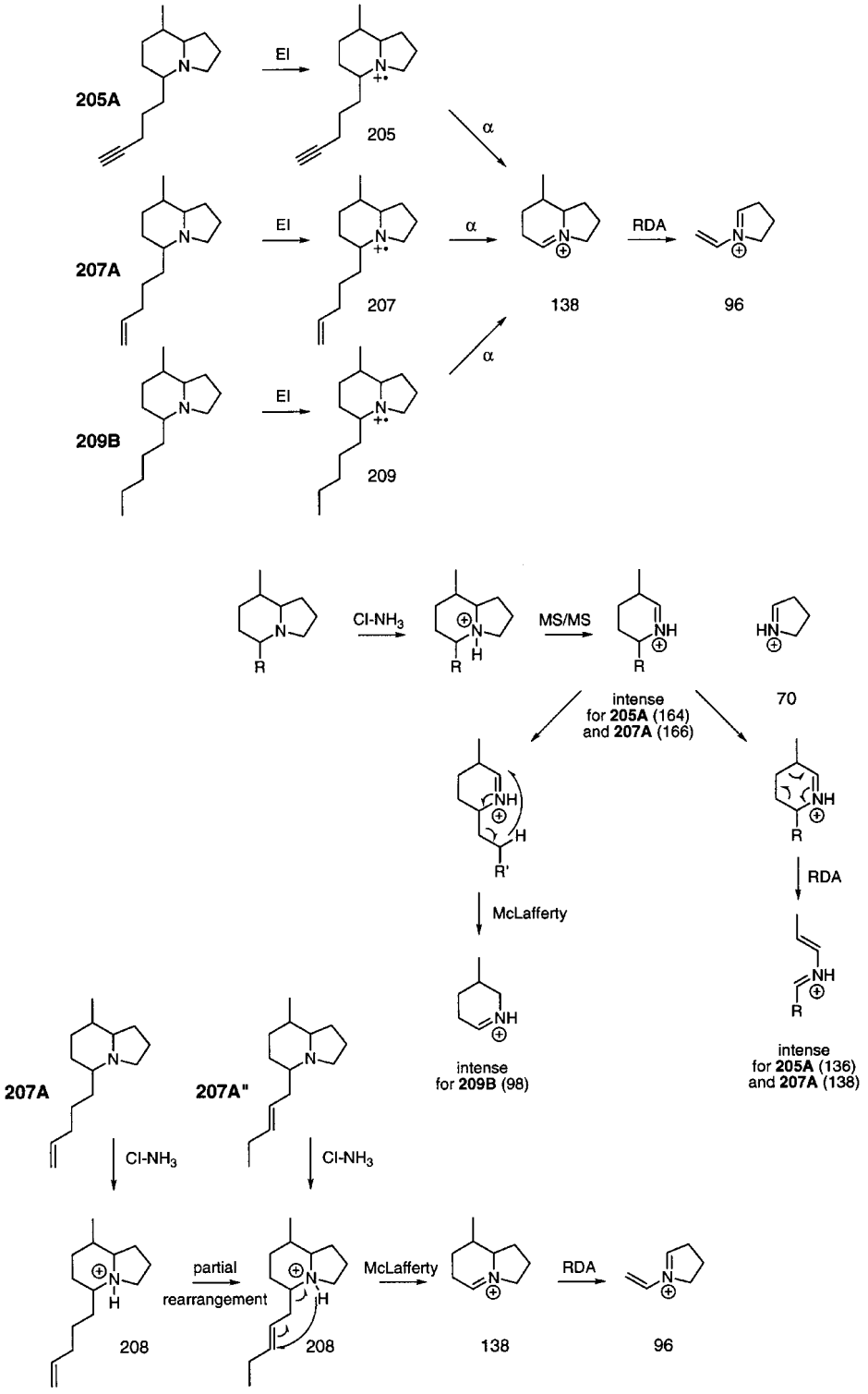


Figure 5. CI-MS/MS spectrum of (5*Z*,9*Z*)-3-*n*-butyl-5-*n*-propylindolizidine (5*Z*,9*Z*-**223AB**).



Scheme 6. Proposed EI and CI-MS/MS fragmentation pathways for three 5-substituted 8-methyl-indolizidines from frog skin, **205A, 207A** and **209B**.

monocyclic alkaloids, the odd-mass peaks often include ions two Da. higher at *m/z* 83, 97 and 111. Fragmentation of *cis*-**219A** (*cis*-2,5-diallyldecahydroquinoline), with an allylic substituent α to the N-atom, is proposed in Scheme 3 to illustrate that, after a McLafferty-like rearrangement, the ion produced is the same as the base peak of the EI spectrum and, thus, the two spectra are similar. The CI-MS/MS spectrum of *cis*-**219A** is shown in Fig. 3(b). The odd-mass peaks are minor. The ion at *m/z* 136 results from a loss of propene by a McLafferty-like rearrangement from the base peak at *m/z* 178, as is the case for the EI mass spectrum (*m/z* 136 is the base peak in the daughter spectrum on EI-MS/MS of *m/z* 178). The ion at *m/z* 108 on NH_3-CI-MS/MS of *cis*-**219A** could arise from the ion at *m/z* 136 as proposed

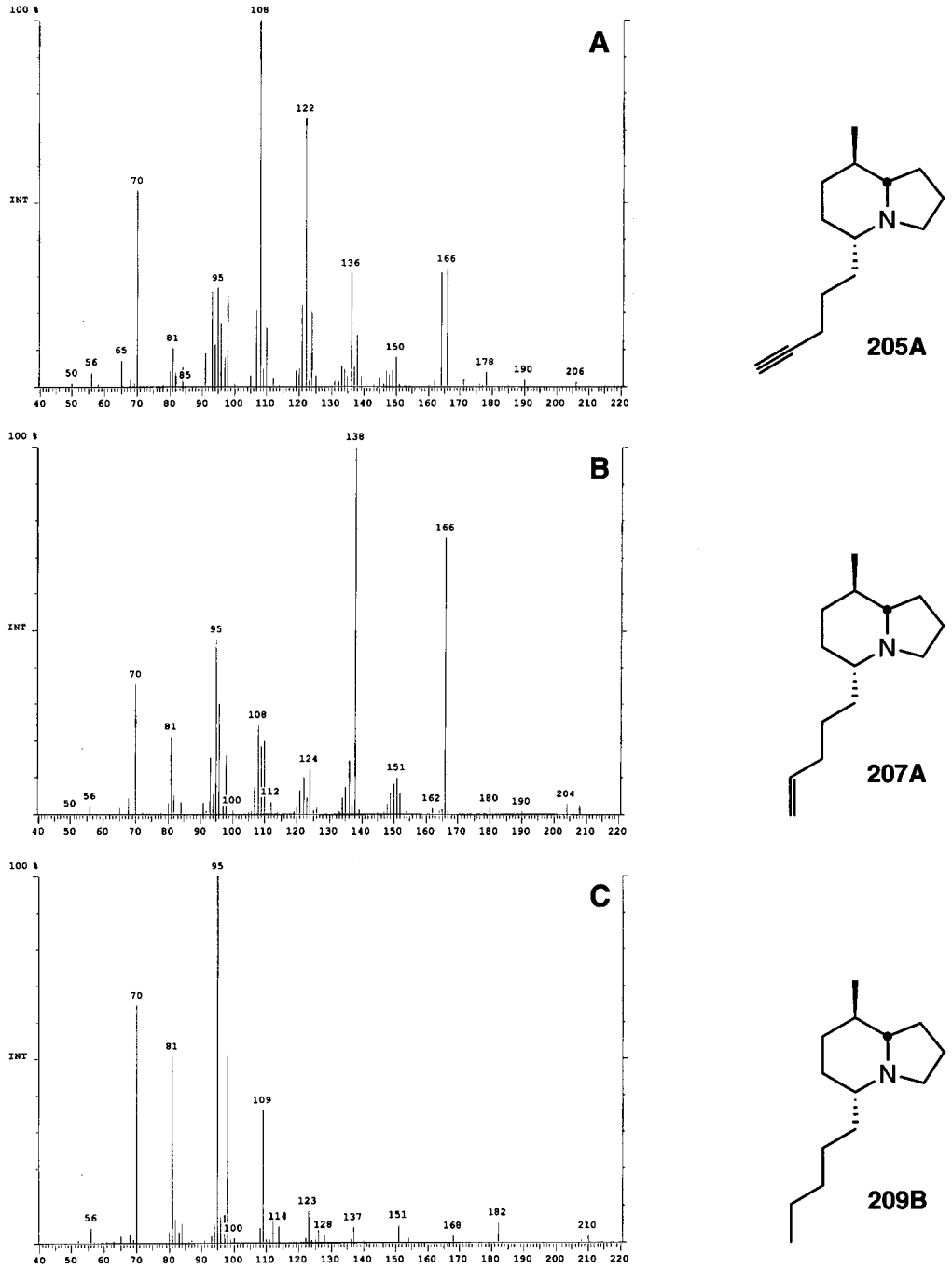


Figure 6. CI-MS/MS spectra of three 5-substituted 8-methylindolizidines isolated from frog skin, **205A** (a), **207A** (b), and **209B** (c).

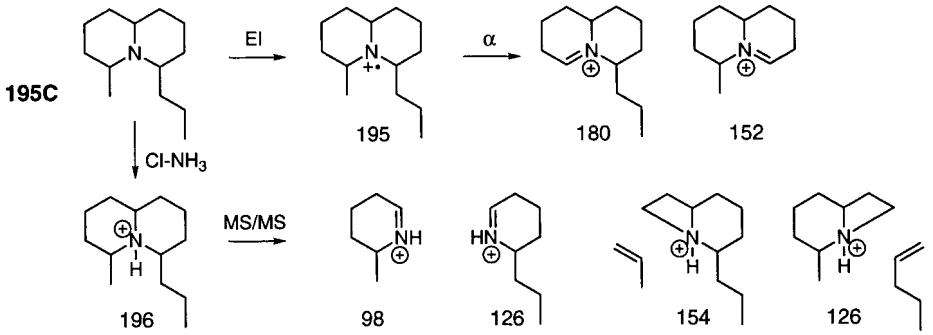
(*m/z* 108 is also a major peak in the EI-MS/MS of *m/z* 136). *trans*- and *cis*-Fused decahydroquinolines yield very similar spectra (data not shown).

3,5-Disubstituted pyrrolizidines

It is proposed that these pyrrolizidines fragment on NH_3-CI-MS/MS as shown in Scheme 4 for 3-methyl-5-nonylpyrrolizidine. The spectrum is shown in Fig. 4. Two major ions with even masses are present, in this case at *m/z* 84 and 196, each retaining just one ring. In contrast, on EI-MS, two α-cleavage ions are obtained at *m/z* 124 and 236, each containing both rings and formed by the loss of one or the other substituent.

3,5-Disubstituted indolizidines

Such indolizidines are proposed to fragment on NH_3-CI-MS/MS in a manner similar to the pyrrolizidines, as shown in Scheme 5 for 5Z,9Z-**223AB** (3-butyl-5-propylindolizidine). The spectrum is shown in Fig. 5. Two major fragments should arise, corresponding to the loss of one ring or the other but, in this case, both fragments are seen at *m/z* 126. In conjunction with the EI mass spectrum, this conclusively shows that the butyl group is on the five-



Scheme 7. Proposed EI and CI-MS/MS fragmentation pathways for a frog and ant alkaloid 4-methyl-6-*n*-propylquinolizidine (**195C**).

membered ring and the propyl group on the six-membered ring. The ion at *m/z* 154 can be explained by rearrangement loss of a pentene moiety. The usual envelope of odd-mass peaks is also found at *m/z* 81, 95, 109, 123 and 137, although the intensity is relatively low. All four stereoisomers of **223AB** yield very similar spectra (data not shown).

5,8-Disubstituted indolizidines

Such indolizidines exhibit complex fragmentations on NH_3-CI-MS/MS as proposed in Scheme 6. Spectra for three such indolizidines are shown in Fig. 6. The three alkaloids **205A, 207A** and **209B** differ only in the unsaturation of the side chain. All show an ion for the five-membered ring at *m/z* 70. The equivalent ion for the six-membered ring is prominent only for the unsaturated alkaloids (ions at *m/z* 166 for **207A**, and at *m/z* 164 for **205A**, respectively). In the case of **207A**, the peak at *m/z* 166 probably represents two different ions, one with the six-membered ring and the other corresponding to loss of propene. Propene loss can be diagnostic for a terminal double bond in a substituent. In the case of a terminal triple bond, an ion at $[M+H-40]^+$ is generally observed, as is the case for **205A**, for which both ions, *m/z* 166 (loss of propyne) and 164 (the ion from the six-membered ring), are observed (Fig. 6(a)). From the ion with the six-membered ring, two further cleavages can be expected, a McLafferty-like rearrangement and a retro Diels-Alder-like cleavage. The importance of these two pathways varies in the three alkaloids. The intensity of the odd-mass peaks at *m/z* 81, 95, 109, 123, etc., also varies. In addition, **205A** shows the most intense ions at *m/z* 108 and 122 (Fig. 6(a)). A fragmentation pathway for these ions is not easy to propose. A further study including CI-MS/MS/MS of indolizidines and quinolizidines with double and triple bonds is in progress. For **207A**, there must be some double-bond isomerization of the protonated parent molecule to yield an isomer with the double bond allylic to the ring (Scheme 6, Fig. 6(b)). This double-bond isomer then cleaves easily after a McLafferty-like rearrangement to yield the ion at *m/z* 138, and this, in turn, via a retro Diels-Alder cleavage, yields the ion at *m/z* 96. The protonated molecular ion without isomerization cleaves 'normally' to produce ions at *m/z* 166 and 70. Alkaloid **205A** with a terminal triple bond would not be expected to undergo such a facile isomerization. An isomer of **207A**, the 5,8-disubstituted indolizidine **207A″**, is known to have an internal double bond in the side chain.[10] Analysis by CI-MS/MS shows a spectrum very similar to the EI spectrum (Scheme 6) establishing an allylic position as the most probable for the unsaturation in **207A″**. In contrast to the CI-MS/MS spectra, the EI spectra of these four 5,8-disubstituted indolizidine alkaloids are very simple, being dominated by the α-cleavage leading to the base peak at *m/z* 138 (Scheme 6). Diastereomers of 5,8-disubstituted indolizidines have not been studied.

4,6-Disubstituted quinolizidines

Such quinolizidines are proposed to fragment on NH_3-CI-MS/MS in a manner similar to the 3,5-disubstituted

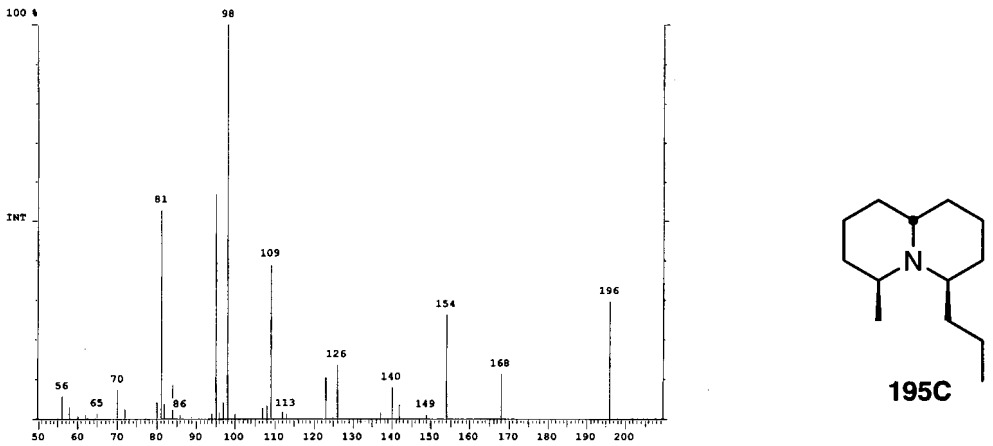
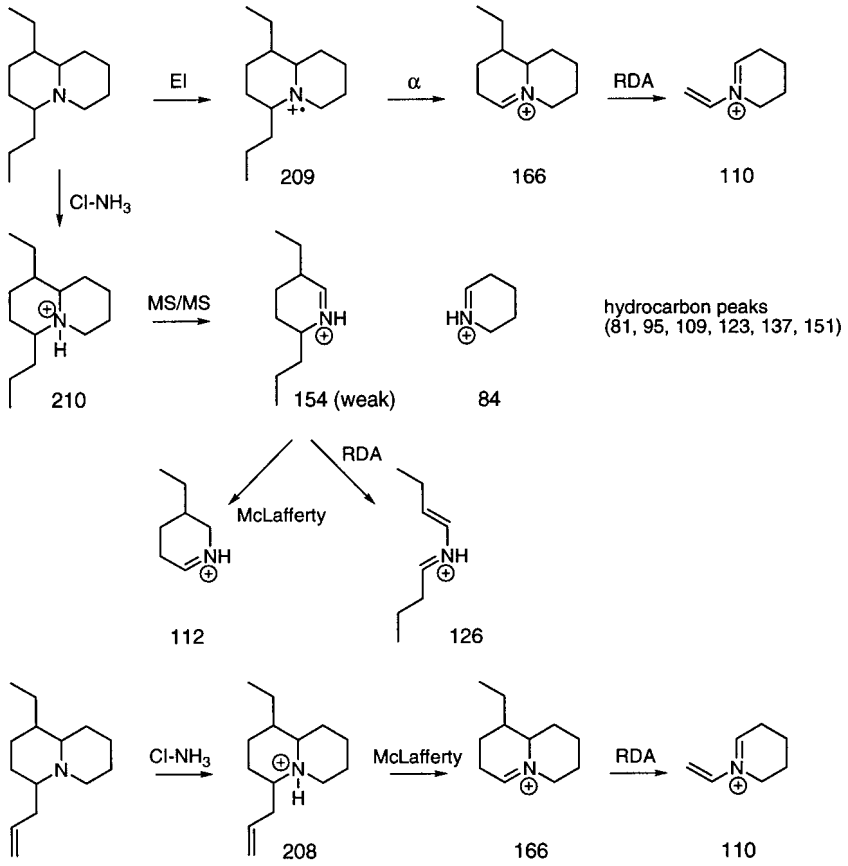


Figure 7. CI-MS/MS spectrum of (6*Z*,10*E*)-4-methyl-6-*n*-propylquinolizidine (**195C**).



Scheme 8. Proposed EI and CI-MS/MS fragmentation pathways for 4-*n*-propyl- and-4-allyl-substituted 2-ethylquinolizidines.

indolizidines, as shown in Scheme 7 for alkaloid **195C** (4-methyl-6-propylquinolizidine). The spectrum is shown in Fig. 7. Alkaloid **195C** exhibits diagnostic ions at *m/z* 98 and 126, which would result from cleavage of one ring, leaving the other with the positive charge. Two other ions, one at *m/z* 154 and the other again at *m/z* 126, are expected as shown in Scheme 7. There is a loss of 28 Da to yield the ion at *m/z* 168. Fragmentations equivalent to those yielding ions *m/z* 154 and 168 are observed also for indolizidine **223AB** (Fig. 5), from the parent ion at *m/z* 224 yielding *m/z* 182 and to 196. There is also the usual envelope of odd-mass ions at *m/z* 81, 95, 109, 123 and 137. All four diastereomers of alkaloid **195C** yield very similar spectra (data not shown).

1,4-Disubstituted quinolizidines

Such quinolizidines are proposed to fragment on NH_3-CI-MS/MS in a complex manner similar to the 5,8-disubstituted indolizidines as shown in Scheme 8 for 1-ethyl-4-propylquinolizidine. The spectrum is shown in Fig. 8(a). 1-Ethyl-4-propylquinolizidine displays *m/z* 84 as the most prominent even-mass ion. The corresponding ion at *m/z* 154 is weak, but the fragment ions at *m/z* 112 and 126 could arise by cleavages with rearrangements from *m/z* 154. A loss of 28 Da is also present, as well as the usual envelope of odd-mass ions at *m/z* 81, 95, 109, 123, 137 and 151. 1-Ethyl-4-allylquinolizidine, with an allylic double bond, cleaves readily by a McLafferty-like rearrangement to give the ion at *m/z* 166 and, thus, the CI-MS/MS spectrum of this compound (Fig. 8(b)) is similar to its EI spectrum. Diastereomers of 1,4-disubstituted quinolizidines have not been studied.

Azabicyclo[5.3.0]decanes

A new class of izidine alkaloids has recently been identified and NH_3-CI-MS/MS analysis played a major role in the structural elucidation.[1,11] EI-MS did not allow discrimination between a 4,6-disubstituted quinolizidine system and an isomeric disubstituted 7-5 ring system. In contrast NH_3-CI-MS/MS, as shown in Scheme 9, clearly indicated that the alkaloid **275A** was an azabicyclo[5.3.0]decane, a conclusion subsequently confirmed by synthesis.[11] The spectra of **275A** and perhydro-**275A** are shown in Fig. 9. The major even-mass fragment ions of alkaloid **275A** are at *m/z* 112 and 192, containing the seven- and the five-membered ring, respectively. The presence of the terminal triple bond in **275A** is demonstrated by an ion at *m/z* 236 representing the loss of propyne from the protonated molecular ion, while the ion at *m/z* 234 corresponds to a propene loss, in this case from the seven-membered ring (Scheme 9). The envelope of odd-mass ions is also present. The perhydro derivative of **275A** gives ions at *m/z* 112 and 196, together with odd-mass ions at *m/z* 81, 95, 109 and 123, and it also shows the loss of propene from the protonated molecular ion (*m/z* 280) at *m/z* 238, but not the loss of propyne that is diagnostic for a terminal triple bond.

SUMMARY

Tandem mass spectrometry after NH_3 chemical ionization

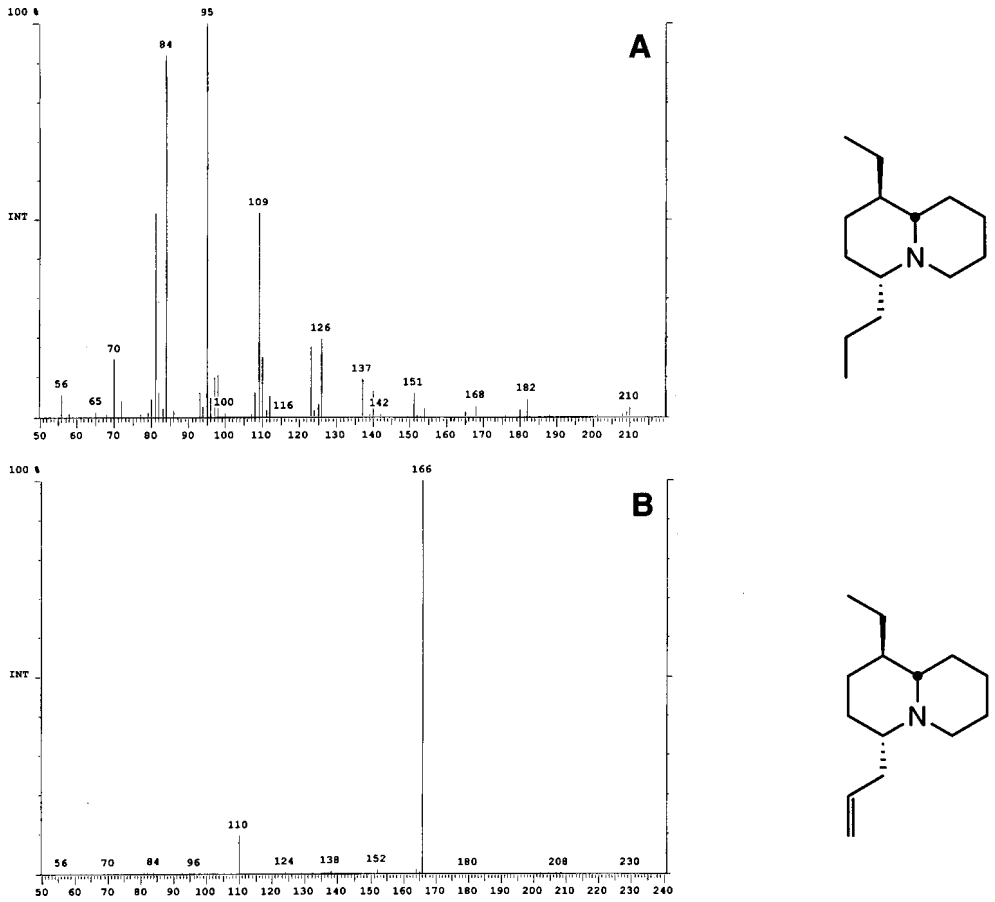
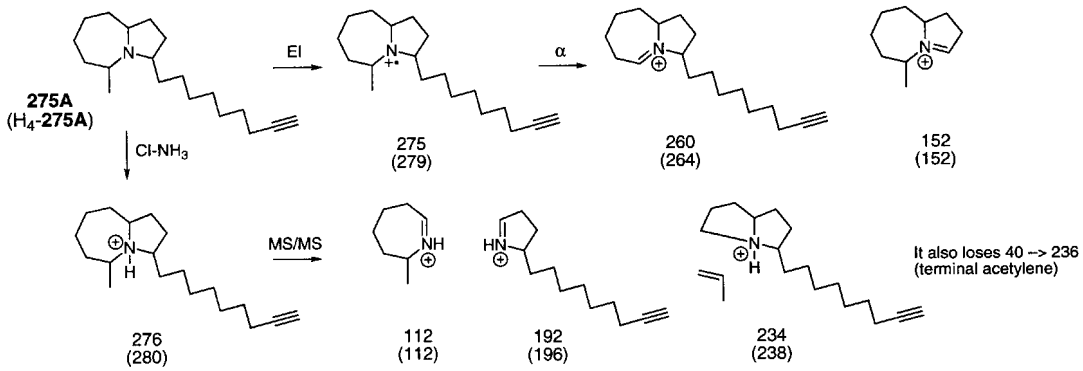


Figure 8. CI-MS/MS spectra of 1-ethyl-4-*n*-propylquinolizidine (a) and 1-ethyl-4-allylquinolizidine (b).

appears to represent a powerful technique for organic structural determination, but has been employed mainly in analysis of compounds in complex mixtures. In spite of the importance of CI-MS/MS and the use of standards as an analytical technique to detect the presence of a single component in complex mixtures, this constitutes only one of the applications open to this technique. Since the NH_3-CI-MS/MS spectra are in general very different from those obtained under EI conditions, the information obtained is usually new and complementary to EI-MS.

In this paper we present spectra of several classes of monocyclic and bicyclic alkaloids and propose fragmentation pathways that could serve as simple models for the investigation of alkaloids of unknown structures with NH_3-CI-MS/MS. In the case of the 4,6-disubstituted quinolizidine **195C**, the proposed structure was confirmed by this technique.[12] In the case of the unprecedented structure of the 3,5-disubstituted azabicyclodecane **275A**, CI-MS/MS was also the key to solving the structural puzzle and to provide the data to warrant synthesis.[11] These two examples illustrate the value of CI-MS/MS for structure elucidation and confirmation. Another example is provided by alkaloid **207A**″, known to have an internal double bond in the side chain. The exact location of the double bond in **207A**″ was



Scheme 9. Proposed EI and CI-MS/MS fragmentation pathways for a frog skin alkaloid 3-(*n*-nonyn-8-yl)-5-methylazabicyclo[5.3.0]decane (**275A**) and its tetrahydro analog, H_4-**275A**.

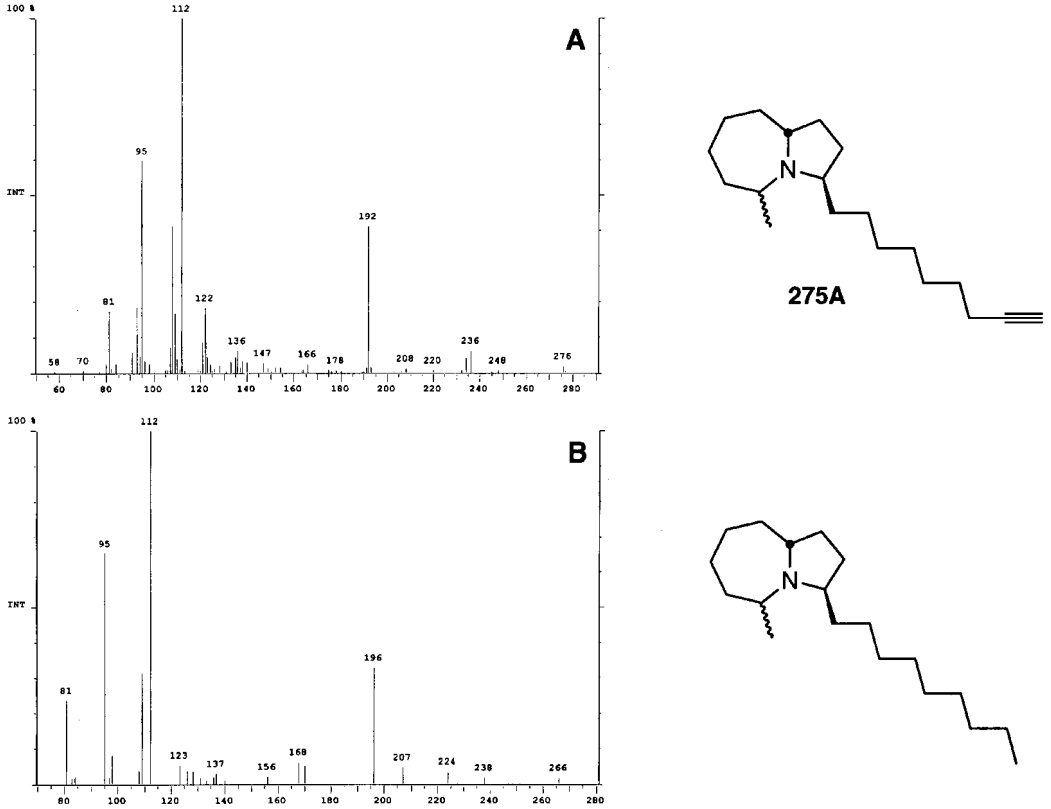


Figure 9. CI-MS/MS spectra of 3-(*n*-nonyn-8-yl)-5-methylazabicyclo[5.3.0]decane (**275A**) (a) and 3-*n*-nonyl-5-methylazabicyclo[5.3.0]decane (b).

unknown and very difficult to assert without isolation and study by NMR. However, CI-MS/MS indicates an allylic position for the double bond in **207A**″. Our study of CI-MS/MS is being extended to other alkaloids, including tricyclic alkaloids and alkaloids having other functional groups (—OH, =O, etc.) in order to provide a broad basis for routine use of CI-MS/MS in structural elucidation as a complement to EI-MS. One of several problems is that alkaloids with a hydroxyl group tend to cleave only by loss of water from the protonated parent molecule (data not shown). Thus, no more information could be extracted on CI-MS/MS with our current ion trap quadrupole instrument. In order to study the fragmentation of the $[M+H-H_2O]^+$ ion, another step of MS, i.e. MS/MS/MS, is required. The potential of tandem mass spectrometry in the CI mode will only be fully realized when fragmentations from more steps of MS, at least CI-MS/MS/MS, can be analyzed.

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