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CHARACTERIZATION OF ALKALOIDS AND OTHER SECONDARY METABOLITES BY MULTIPLE STAGE MASS SPECTROMETRY[1]

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ABSTRACT.—Tandem mass spectrometry (ms/ms) is reviewed with illustrations of its applications drawn from the natural products area. The various types of ms/ms scans—daughter, parent, and neutral loss—are described together with their favorable effects on ms detection limits and speed of analysis. The applications of ms/ms in conjunction with desorption ionization for the identification of quaternary alkaloids are illustrated. Isomer identification including energy resolved forms of ms/ms, the use of negative ions, and aspects of fragmentation patterns in ms/ms are all presented. Applications of tandem mass spectrometry to mapping alkaloid distribution, detection of new trace alkaloids, and quantitative analysis are covered.

The analysis of natural products has been facilitated by the emergence of multiple stage mass spectrometry (1). A development from metastable ions (2), tandem mass spectrometry, or ms/ms, separates the ionization process from fragmentation and increases the information obtainable from a sample. Early experiments (3) utilized a reversed geometry mass spectrometer, also known as a mass-analyzed ion kinetic energy spectrometer (mikes), but currently ms/ms experiments are performed on a variety of devices including multiple quadrupole instruments (4), hybrids consisting of both sector and quadrupole analyzers (5), and Fourier transform mass spectrometers (6). Ms/ms procedures minimize sample treatment prior to analysis (3) without sacrificing structural specificity or sensitivity, the hallmarks of mass spectrometry. It is possible to make positive identifications of specific mixture components, even when intact plant material is examined.

Ms/ms has often been compared with gc/ms, and the comparison is apt when the simplest type of two-dimensional experiment is considered (7,8). In this particular procedure, a spectrum of daughter ions arising from a selected precursor ion is recorded. The precursor ion, which serves as a surrogate for the neutral compound, is separated from other components of the mixture by the first stage of mass analysis. This separation procedure has disadvantages compared with chromatographic separation, especially for isomeric molecules, but it also has distinct advantages. These include decreased analysis times (9), continuous availability of all mixture components for examination (10), and improved limits of detection (11). This last advantage arises chiefly by suppression of interference, or chemical noise, and Figure 1 illustrates the remarkable improvement in signal-to-noise achieved in tandem mass spectrometry over a single stage of mass analysis (12). Components of mixtures that are obscured in the mass spectrum are clearly revealed in the ms/ms daughter scans, as shown here. Tandem mass spectrometry makes possible the identification, with minimal sample work-up, of compounds that could previously be identified only after extraction, chromatography, centrifugation, and/or derivatization.

This paper will examine ms/ms as it applies to the characterization of alkaloids and other secondary metabolites in plant material. Multiple stage ms combines mixture separation and component identification in an integrated system (13) and provides a variety of scan types that can be selected to access particular types of information. In addition to the several ms/ms scan procedures, the ionization method itself can be chosen to maximize information obtainable from compounds of particular types. Isobutane

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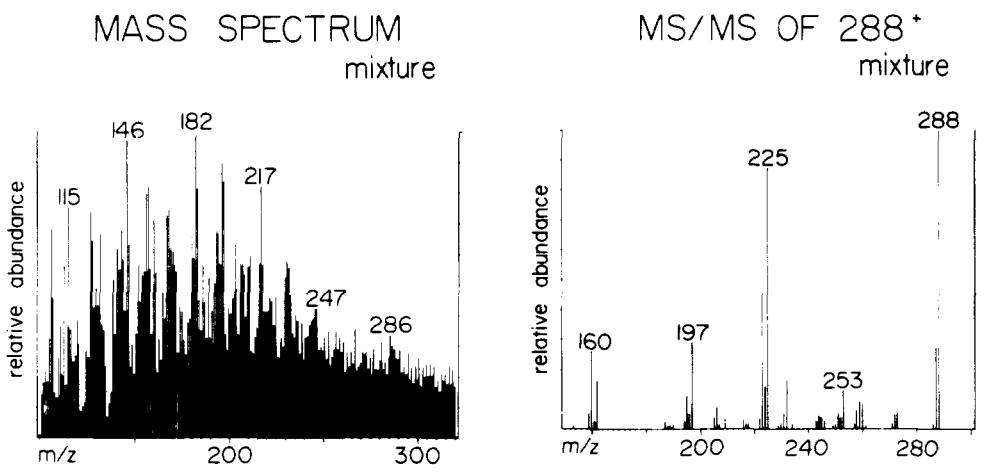


FIGURE 1. Electron impact mass spectrum of a complex mixture compared to the ms/ms daughter spectrum of 288^+ of the same mixture. (From reference 12)

chemical ionization, a selective and gentle method of ionizing basic compounds by proton transfer, is often useful in enhancing alkaloid signals relative to those due to plant matrix (14). The newer techniques of desorption ionization (15), which make possible the analysis of thermally labile or involatile samples, including quaternary alkaloids, are applicable with ms/ms, and their use will be illustrated together with that of the more traditional procedures of electron ionization and positive and negative chemical ionization.

Reducing the effort of the experimenter is not the only feature that makes the ms/ms techniques interesting for natural product analysis. Equally important is the fact that the amount of sample required is drastically reduced. In an early experiment, analysis of a few grams of ground, freshly cut poison hemlock was used to detect the alkaloid coniine (16), while more recently, much smaller size samples have been used to detect new, as well as previously known, alkaloids (17). Detection limits in these types of experiments are 0.001% dry weight, or less.

Tandem mass spectrometry can be applied to natural product chemistry to answer various questions. Rapid searches and quantitation can be carried out for targeted molecules, such as has been done for mescaline (18). Screens for individual compounds can be run before undertaking extensive isolation procedures, thus saving time and expense. New members of known alkaloid classes can be sought using the newer types of scans that are functional-group specific (19). Labeling studies, employed for determining fragmentation mechanisms, are facilitated by ms/ms, because the labeled analog can be examined, even when it is not available in a chemically or isotopically pure state (20). In addition to these capabilities, ms/ms can be used to determine alkaloid distributions in a plant. For example, examination of small sections of plant material taken from whole coca plant has been used to map the relative concentration of cocaine and cinnamoylcocaine in the various tissues (9,21) (Table 1). In another application, the direct sampling capability available with ms/ms has been used to avoid incorrect deductions associated with sample modification that may occur during isolation (3,9). Of course, ms/ms spectra themselves can include signals due to artifacts that arise after sample introduction into the mass spectrometer, but these seldom cause difficulties. In summary, the proven capabilities of ms/ms in trace analysis, metabolite identification (22), and mechanistic studies (23) should all enhance the study of alkaloids and other secondary plant metabolites.

TABLE 1. Distribution of Cocaine and Cinnamoylcocaine in *Erythroxylum coca* Lam. from the Tingo Maria Variety

Plant part	Cinnamoylcocaine (%)	Cocaine/ Cinnamoylcocaine
leaf, center	6	15
leaf, stem	6	15
berry, side	68	0.5
berry, outside	33	2.0
berry, stem	50	1.0

THE MS/MS SPECTRUM.—The mass spectrometer is usually thought of as an analytical device, not as a separator, yet the two functions are intimately connected. Large mass spectrometers, known as calutrons, have been used for forty years to separate and isolate macroscopic amounts of particular elements (24). If two mass spectrometers are linked in tandem, it is possible to employ the first as a separator and the second as an analyzer to perform direct analysis of mixtures (25). Ions representative of a particular constituent of the mixture emerge from the first mass spectrometer and pass into the source of the second. Here, some transformation, usually collision-induced dissociation (cid) (26), is effected, and the ionic products of this reaction are mass-analyzed to provide a spectrum (the ms/ms daughter spectrum) characterizing the particular ion selected initially. This type of scan, a daughter scan, is the one that is most widely used to characterize specific compounds. Such a spectrum is a fingerprint of the primary ion and hence of its neutral counterpart.

In addition to the daughter scan just described, other scan modes can be used for analysis by the ms/ms technique, as diagrammed in Figure 2. In a parent scan, the first analyzer is scanned while the second analyzer is held constant, giving a spectrum of all the reactant ions leading to a particular fragment ion (10,27). In a neutral loss scan, both analyzers are scanned simultaneously. Under appropriate conditions, this can provide a molecular weight profile of all those ions in the original mixture which fragment via loss of a particular neutral fragment (10). Such fragments are often characteristic of particular functional groups.

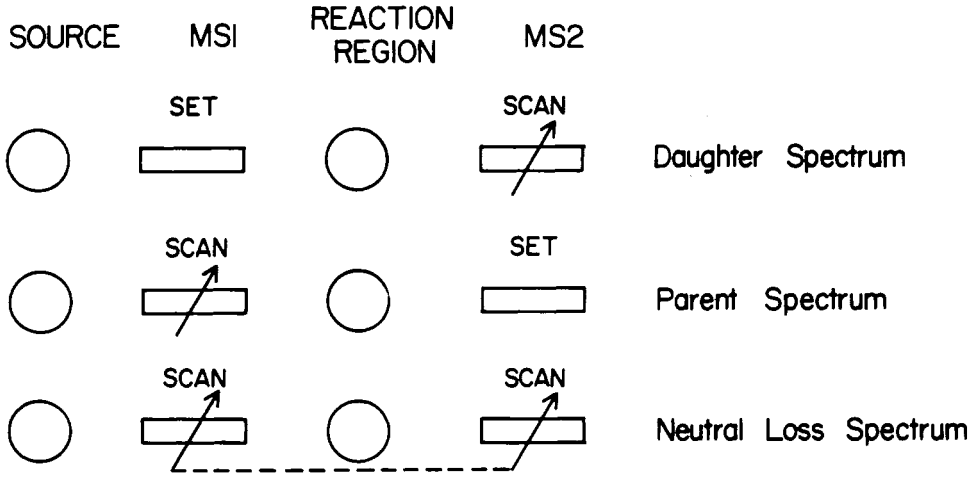


FIGURE 2. Scan modes made available with tandem mass spectrometry.

REACTION MONITORING.—Monitoring of a single reaction, rather than a full spectrum, provides the highest sensitivity obtainable with ms/ms. This is done by scanning neither mass analyzer but simply by monitoring the ion current due to a selected reaction as a function of time. Multiple reaction monitoring, a variant of single reaction monitoring, increases the specificity of detection (28) and is usually the procedure of choice. Because analysis time can be concentrated on those regions of the data array that are richest in information, these methods maximize sensitivity, although they lose specificity by limiting the data space examined. The *Erythroxylum coca* mapping experiment (see above) (9,21) employed single reaction monitoring, the loss of benzoic acid from protonated cocaine being monitored to characterize this alkaloid.

Another, quite distinct, use to which multiple reaction monitoring methods can be put involves the monitoring of several different molecular species, in effect, simultaneously (13). This might be done when the sample is changing in composition with time, say during the course of a chemical reaction, or it might be done to avoid instrument fluctuations when accurate quantitation of two or more species is sought (13).

Table 2 outlines some of the options that exist in selecting the type of reaction used to characterize the separated ions (10). Spontaneous dissociation of metastable ions often involves elimination of simple neutral molecules (29). Cid (30), however, is more useful in structural determination because the relative intensities of the peaks increase and many more reactions occur (29,13). Cid forms a reproducible and characteristic fingerprint of the fragment ions (9) by exciting the reactant ions in a glancing collision with a target gas. Still little used in analysis, but of increasing importance, are processes in which the number of charges on the ion is changed either by stripping electrons or by charge exchange (26). Reactive ion/molecule collisions in which adduct ions are generated can also be expected to be increasingly used in ms/ms (31). This type of process should be contrasted with the fragmentations that have been utilized so extensively up to now.

TABLE 2. Reactions Utilized in Tandem Mass Spectrometry

1.	$m_1^{+*} \mapsto m_2^+ + m_3$	Metastable Ion Dissociation
2.	$m_1^+ \overset{N}{\mapsto} m_2^+ + m_3$	Collision Induced Dissociation[a]
3.	$m_1^+ \overset{N}{\mapsto} (m_1N)^+ \mapsto m_2^+ + m_3$	Reactive Collision
4.	$m_1^+ \overset{N}{\mapsto} m_1^{2+} + e^-$	Charge Stripping
5.	$m_1^- \overset{N}{\mapsto} m_1^+ + 2e^-$	Charge Inversion
6.	$m^{2+} \overset{N}{\mapsto} m^+ + N^+$	Charge Exchange

[a]Also known as collision activated decomposition.

IONIZATION IN THE FIRST MASS SPECTROMETER.—The ionization method of choice in ms/ms should give the minimum number of ions for each neutral component and not cause molecular rearrangements (13). The method need not be universal, but if selective, efficient ionization of the analyte is obviously required. Of the conventional ionizing techniques, chemical ionization (ci) better satisfies these requirements than electron ionization (ei) (13) because molecular ions are more abundant and less fragmentation and far less rearrangement occur with ci than with ei.

Because ci is a technique in which ions are formed in ion-molecule collisions, for example, those involving protonation or hydride abstraction as the primary process (32), different reagent ions can be used to alter the energy transfer associated with ionization

or to change the selectivity of the method. Chemical ionization can be made more selective by using weak gas-phase acids such as $C_4H_9^+$ or H_3O^+ for protonation, or by negative chemical ionization, for example, using OH^- as reagent for proton abstraction or Cl^- as an attachment reagent (10,27,33). Comparison of the ci mass spectra of opium using isobutane and water as reagent gases shows that the base peak, corresponding to a fragment ion at *m/z* 220 with the narcotine composition, is the same in the two spectra, but the relative intensities of the other peaks vary (34). Negative ci is often less subject to interferences due to ionization of other matrix constituents than is positive ci (35). Pyrrolizidine alkaloids have been studied using negative ion chemical ionization with hydroxide as the reactant ion (36). The production of an intact ion from the acid was reported as well as other structural features. Information obtained through negative and positive chemical ionization is often complementary, each giving valuable structural information (37,38).

There is a particular reason for preferring negative ionization over positive which goes beyond the selectivity of the two processes. As illustrated in Figure 3 for a simple aromatic acid, one can obtain *two* distinct and informative ms/ms spectra by dissociating a selected anion (13). One spectrum is a conventional display of anionic fragments. The second represents cationic fragments generated in those collisions providing sufficient energy to strip two electrons from the parent ion. Both spectra have comparable signal strengths within a factor of three in the high collision energy regime.

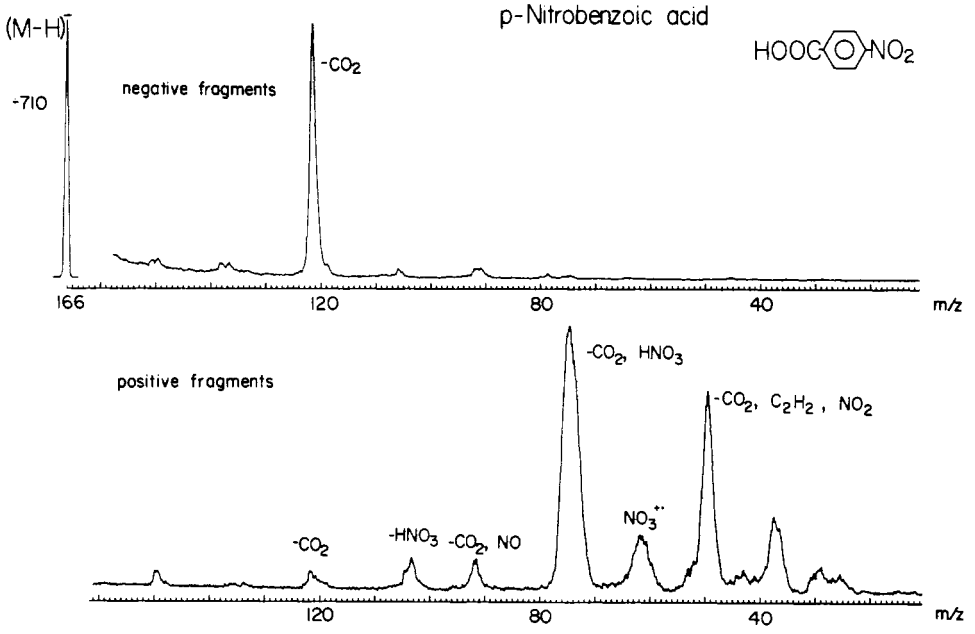
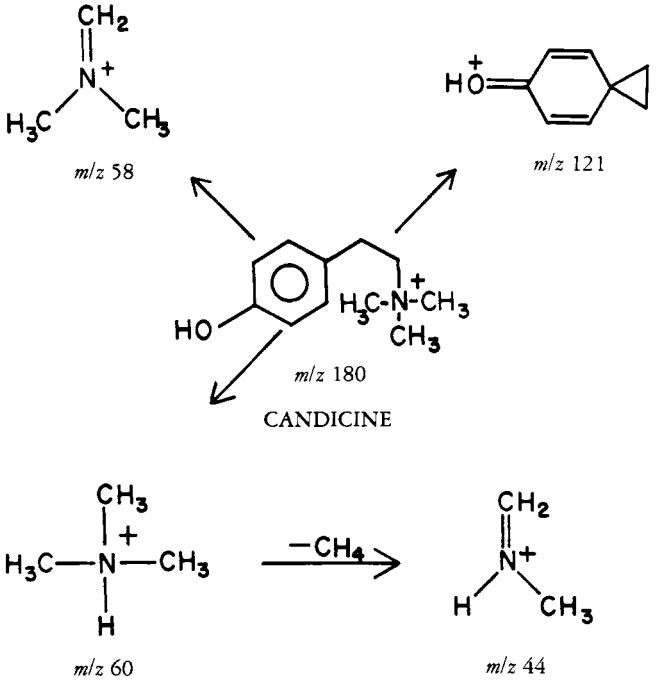


FIGURE 3. Ms/ms daughter spectra of positive and negative ions arising from the $(M-H)^-$ ion of p-nitrobenzoic acid.

Electron ionization (ei) is often undesirable for ms/ms determinations of targeted compounds in mixtures because 70 eV electrons produce many primary ions from most compounds in addition to the molecular ion (9). In the cases of some aromatic compounds, however, electron impact is quite satisfactory for ms/ms work (10,38). Methoxyhalobiphenyls have been studied using electron impact mass spectrometry in conjunction with collisionally activated dissociation and also metastable ion fragmentations. The resulting daughter spectra allow structural identification through identification of fragmentation pathways (39).

IONIZATION TECHNIQUES FOR SPECIAL CASES.—When a sample is thermally labile or nonvolatile, desorption ionization (di) using energetic ion, atom, or laser beams becomes the procedure of choice (8,10,40). Desorption methods circumvent the requirement for vaporization (15) while providing both molecular weight information and a structurally diagnostic set of fragment ions (41,42). For mixtures, as well as for pure compounds, ms/ms avoids interferences from solvent molecules in the di mass spectra and reduces the background noise, sometimes a characteristic of these spectra. Desorption methods are particularly successful in ionizing precharged species, including intact cations of quarternary alkaloids (41). As with other ms/ms techniques, di ms/ms shows much less chemical noise than do the corresponding di mass spectra (43).

To illustrate these capabilities, consider the quaternary cactus alkaloid, candicine. High quality ms/ms spectra of candicine are obtained for a variety of cactus samples when examined by laser desorption (ld) in a ci plasma (41). The major ions, their probable origins and structures are illustrated in Scheme 1. The reactions involved are suggested on the basis of known unimolecular gas-phase chemistry. Figure 4 displays the ld-ci ms/ms spectrum of *m/z* 180 derived from candicine chloride (41). The dominant peak in the spectrum (*m/z* 60) corresponds to formation of protonated trimethylamine. The next most abundant peak (*m/z* 121) is generated by scission of the C-N bond, probably with formation of a stabilized phenonium ion. Also evident at low mass are ions characteristic of protonated amines (41).



SCHEME 1

Referring again to Figure 4, the spectrum of *m/z* 180 from the crude cactus extract of *Trichocereus pasacana* is shown. The plant material was extracted with EtOH, and the residue was partitioned between $CHCl_3$ and H_2O at pH 10. The H_2O layer was freeze-dried, and the residue was reconstituted in MeOH and H_2O for mass spectral analysis. Comparison of the two spectra confirms the presence of the quaternary alkaloid in the cactus.

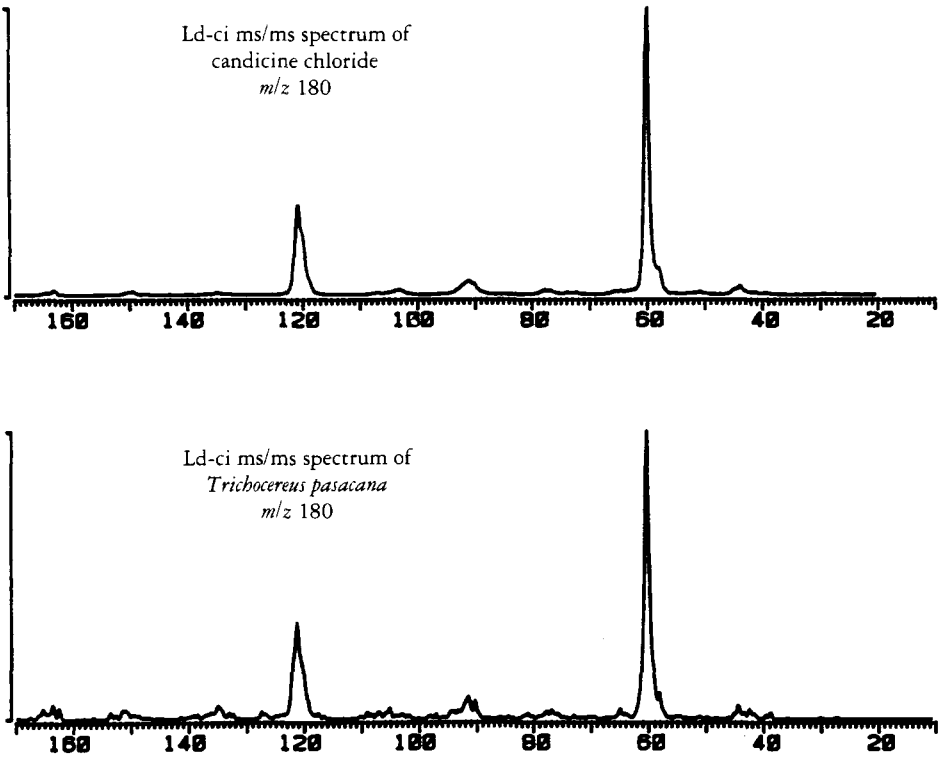


FIGURE 4. Laser desorption chemical ionization ms/ms spectrum of *m/z* 180 from candicine chloride and *m/z* 180 from the crude cactus extract of *Trichocereus pasacana*. (From reference 41)

Two related alkaloids, both homologs of candicine, were charcterized using another form of desorption ionization, secondary ion mass spectrometry (sims) (33,41,44). The fragmentations observed in the sims spectrum indicated the presence of two isomers in *Coryphantha greenwoodii*. These compounds, whose structures were confirmed by synthesis, are *N,N,N*-trimethyl-4-methoxyphenethylamine chloride (*O*-methylcandicine) and *N,N,N*-trimethyl-β-methoxyphenethylamine (coryphanthine), which differ only in the position of the methoxy substituent. The ms/ms spectra (45) of the intact cations are compared in Figure 5. The isomers can be readily distinguished by examining the relative abundance of 135^+ as well as the ratio of $103^+/105^+$. In a similar way, ms/ms has been used to study carnitine hydrochloride, a compound unusual in that it undergoes partial methylation during analysis. To determine the site of methylation, the ms/ms spectra of the carnitine cation 162^+, Figure 6, and that of the methylated analog at *m/z* 176 were compared (45). Parallel reactions were observed, and the comparison indicates that the methylation occurs at the hydroxyl group. Fast atom bombardment combined with ms/ms can also produce a combination suitable for analysis of many polar molecules of biological importance (46).

Depending on the sample to be analyzed, the above discussion shows that appropriate ionizing conditions can be chosen to obtain the desired information (13). By combining a desorption ionization source and a computerized fast scanning ms/ms spectrometer, such as a triple quadrupole (4), all the scan modes of ms/ms are made available in conjunction with this powerful ionization method. In addition, one acquires the capability of controlling energy deposited in the desorbed ion (see below) through variation of the collision energy (47).

MS/MS CAPABILITIES.—An examination of *Conium maculatum* L. for the purpose of

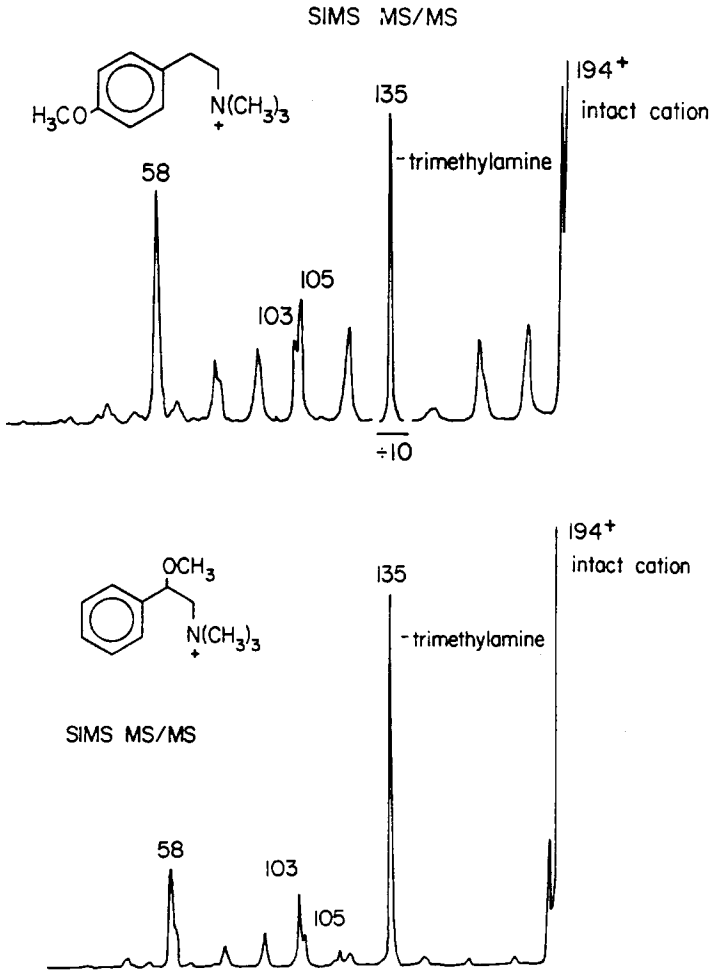


FIGURE 5. Ms/ms spectra of two isomeric alkaloids ionized by secondary ion mass spectrometry. (From reference 45)

detecting the poisonous alkaloid, coniine, illustrates one advantage of ms/ms in the characterization for natural products (16). The mass spectrum of leaf material, Figure 7, shows that the protonated alkaloid of interest gives a peak that is not detectable over background. Yet, its presence is readily detected from the ms/ms spectrum. The fragmentation pattern given in Scheme 2 confirms the presence of the alkaloid.

Another simple example of the capabilities of tandem mass spectrometry is provided by a recent investigation of cactus alkaloids. Ms/ms was used to screen a set of related Mexican columnar cactus species for the presence of tetrahydroisoquinoline alkaloids (48). The results, given in Table 3, were obtained using a mass analyzed ion kinetic energy spectrometer (mikes), with ci (48). Measurements of alkaloid distribution in the cactus species serve to classify the plants. Of particular interest is the distinction between isomeric tetrahydroisoquinolines, as illustrated in Figure 8 where *N*- and *C*-alkylation patterns are easily recognized (48). This study also serves to illustrate one additional advantage of ms/ms analysis by demonstrating that the induction of *N*-methyl to l-methyl isomerization occurs during the anion exchange step in the isolation of dimethoxytetrahydroisoquinolines (48).

The samples used for this work were the crude, ground cactus material, an alkaloid fraction, subfractions of which had been purified by chromatography, and synthetic

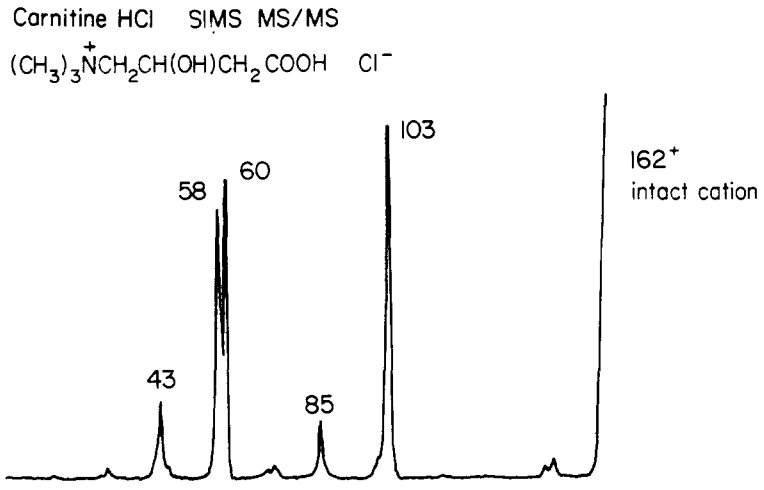


FIGURE 6. Daughter (ms/ms) spectrum of the carnitine cation, after ionization by sims.

standards. The ms/ms spectra taken, using the alkaloid fraction, were of better quality than those obtained with the powdered plant material and were preferred for comparison with the standards. This demonstrates the expected effect of improved spectral quality as sample preparation becomes more extensive (48).

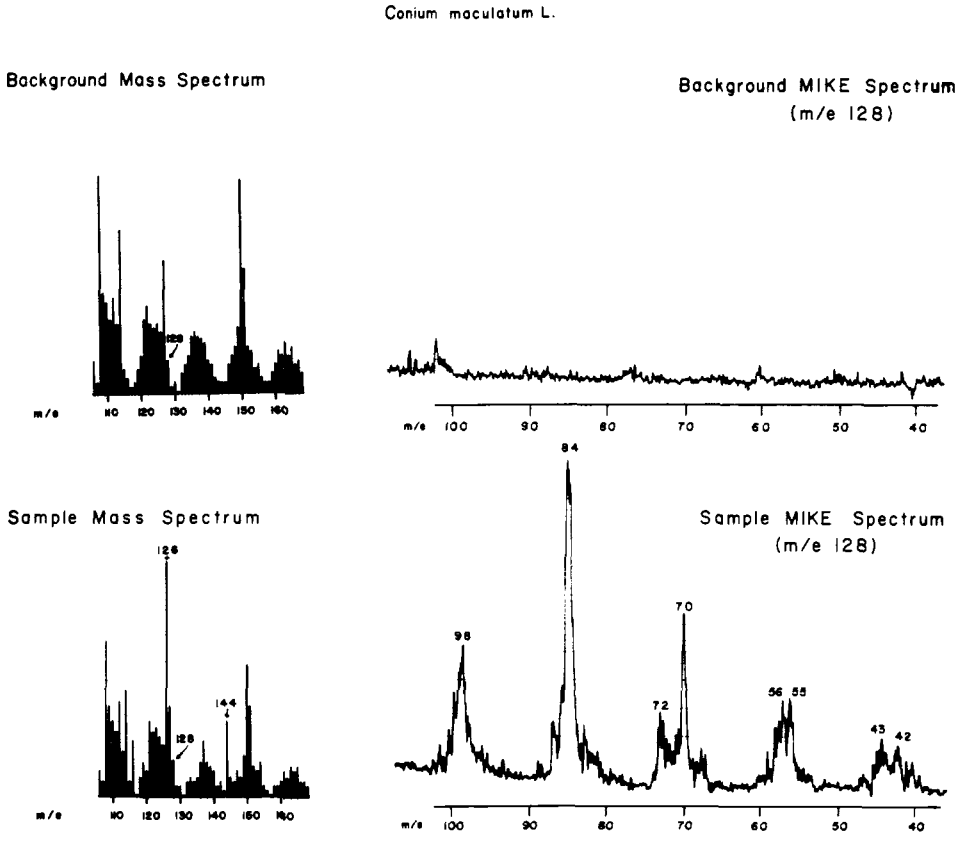
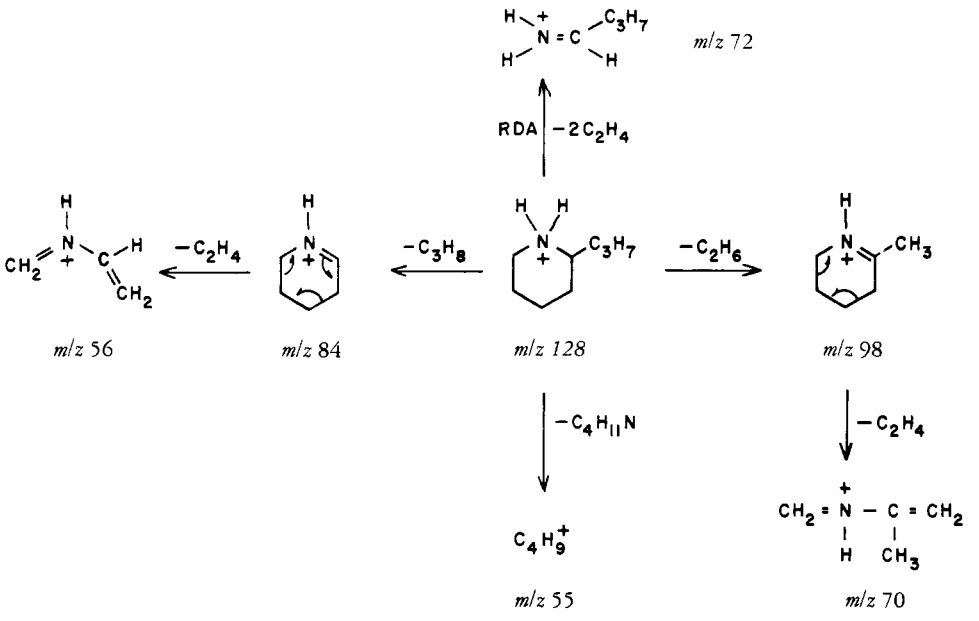


FIGURE 7. Comparison of background mass and ms/ms spectra with the same spectra after introduction of *Conium maculatum* L. (From reference 16. Copyright 1978 by the AAAS)



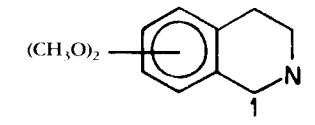
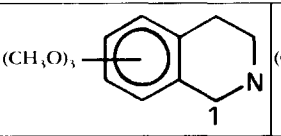
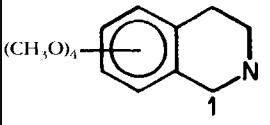
SCHEME 2

In a more recent investigation, the mikes instrument was used to identify seven previously unidentified alkaloids in *Backebergia militaris* (17). Four of the alkaloids, known previously from other cacti, were identified as: *N*-, methyl-3,4-dimethoxyphenethylamine, *N,N*-dimethyl-3,4-dimethoxyphenethylamine, *N*-methylheliamine (2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline), and 1,2-dehydroheliamine. Three novel cactus alkaloids were identified as: 7,8-dimethoxy-3,4-dihydroisoquinoline, 6,7-dimethoxyisoquinoline, and 7,8-dimethoxyisoquinoline and were confirmed by synthesis. In a similar study of the cactus *Pachycereus weberi*, new cactus alkaloids have been encountered (49).

Mass separation does not always yield complete molecular separation because unit mass resolution fails to separate isobars, and even high resolution (13), when available, still fails to separate isomers. Some form of chromatography, chemical derivatization, or chemical work-up of the sample can profitably be combined with ms/ms to solve this problem. Another method that alleviates this problem is temperature profiling carried out by heating the probe (11,50) while continuously recording ms/ms spectra. Temperature profiling can make possible the identification of compounds in the presence of others with the same mass. This procedure has been used to distinguish components of the mushroom *Psilocybe cyanescens*, which contains the psychotomimetic agent psilocybin (11).

An investigation of the saguaro cactus, *Carnegiea gigantea*, to determine whether trace alkaloids were naturally occurring or artifacts, utilized temperature profiling (51). The alkaloid, dehydrosalsolidine, at *m/z* 206 as the protonated molecule, was not seen at low probe temperatures but had high intensity at high temperatures. To ensure that dehydrosalsolidine was a component of the cactus rather than something occurring from the abundant protonated salsolidine ion at *m/z* 208, ms/ms spectra were taken over a range of temperatures. The spectra obtained matched those of synthetic dehydrosalsolidine. The corresponding temperature profile of pure salsolidine showed insignificant *m/z* 206 peaks. Thus, the ions at *m/z* 206 were due to naturally occurring dehydrosalsolidine rather than to artifacts. The alkaloid was subsequently isolated from the cactus where it may be an intermediate in salsolidine biogenesis.

TABLE 3. Chemotaxonomy by Tandem Mass Spectrometry

Species	Compound $(M+H)^+$									
		194	1-CH_3 208	N-CH_3 208	1-CH_3 N-CH_3 222	1-CH_3 238	N-CH_3 238	1-CH_3 N-CH_3 252	N-CH_3 268	1-CH_3 N-CH_3 282
Pachycereus pecten-aboriginum		+	−	+	+	−	+	+	+	+
Pachycereus weberi		+	+	+	+	−	+	+	+	
Pachycereus pringlei		+	−	+	+	−	+		+	−
Carnegea gigantea			+	−	+	+	−	+	−	−
Backebergia militaris		+	+	+	−	−	−	−	−	−
Pachycereus marginatus		−	+	+	−	−	−	−	−	−
Lophocereus schottii		+	−	+	−	−	−	−	−	−
Pachycereus hollianus		−	−	−	−	−	−	−	−	−
Neobuxbaumia euphorbioides		−	−	−	−	−	−	−	−	−

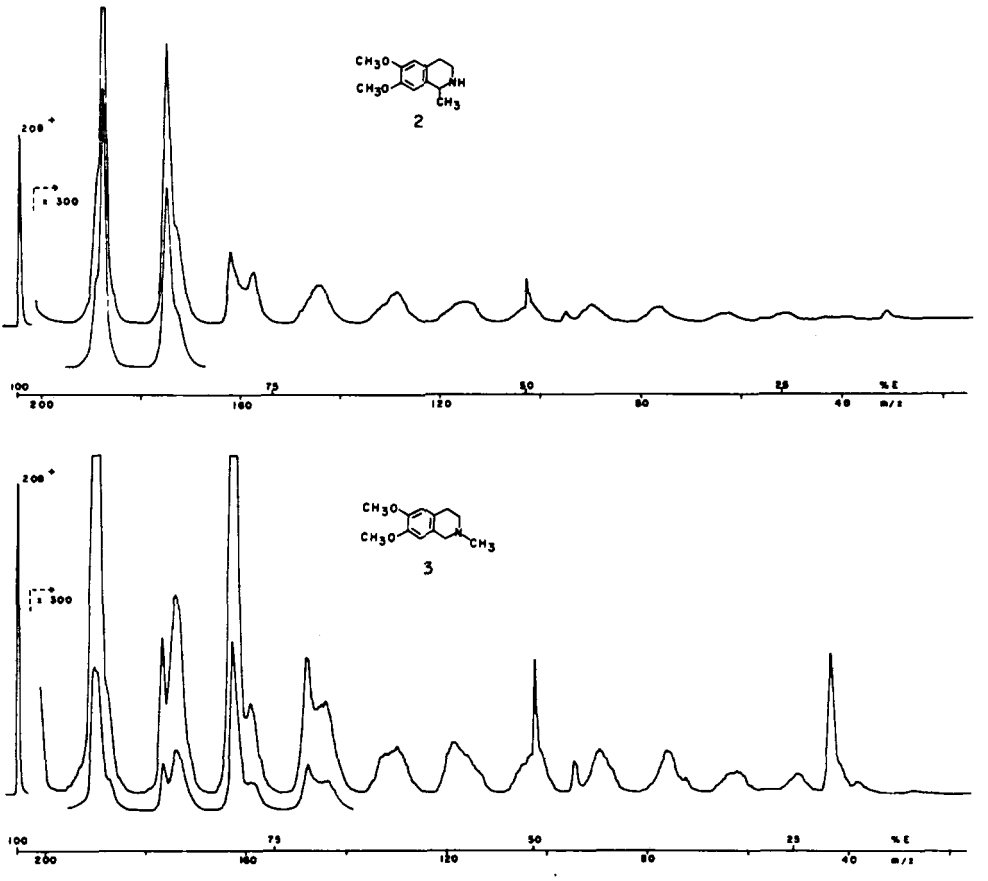


FIGURE 8. Ms/ms spectra used to distinguish isomeric alkaloids. (From reference 48)

Any particular research group or industrial process has as its focus some sub-set of molecules. Given this narrow focus, scan types that can be selected to recognize members of particular chemical classes should be particularly useful. Parent scans have been used in the determination of pyrrolizidine alkaloids in the *Senecio* plant species (52). The protonated molecular ions of the alkaloids of interest yield the common daughter ion $C_8H_{10}N$, *m/z* 120. Hence, a scan for the parents of the ion *m/z* 120 can indicate which pyrrolizidine alkaloids are present and can give an idea of their concentrations.

Searches for particular compounds in complex mixtures can be made by employing daughter scans. This is the best-developed analytical application of tandem mass spectrometry. Identification can then be made by interpreting the ms/ms spectrum or by comparing it with the spectrum of an authentic sample (53). Such a comparison of a spectrum taken of a mushroom sample to that of authentic gyromitrin is shown in Figure 9 (54). The lower spectrum shown was obtained from a total of 1 mg of mushroom. This sample gave spectra for three h, while the spectrum shown was recorded in six min. More recent experiments have reduced even this time requirement by an order of magnitude. Scheme 3 rationalizes the spectrum of protonated gyromitrin.

In a similar fashion, the presence of the maytansinoid antitumor agent, trewiasine, mw 749, can be indicated in extracts by an assay based on multiple reaction monitoring (55). The three major ions that occur in the mass spectrum are *m/z* 689, 659, and 516. By following intensities in these ms/ms spectra as a function of time, detection limits for chromatographic fractions, tissue cultures, and raw plant material can be estab-

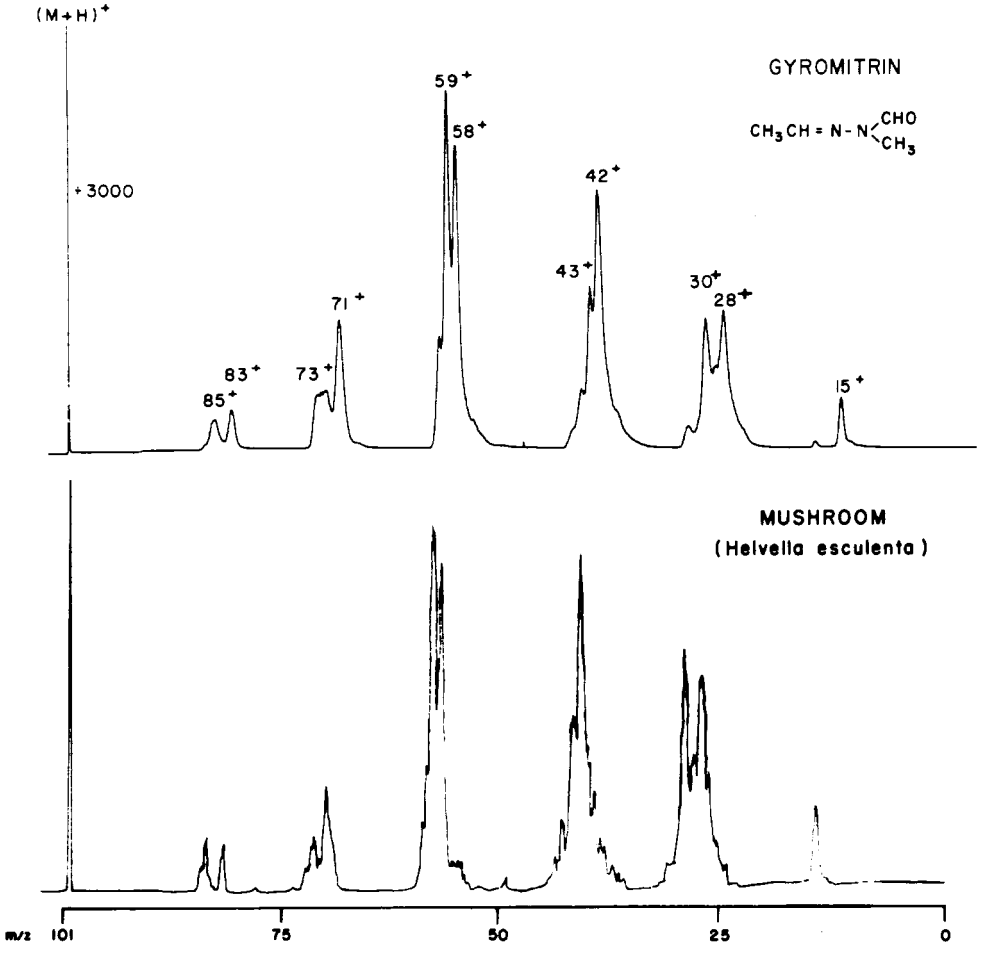
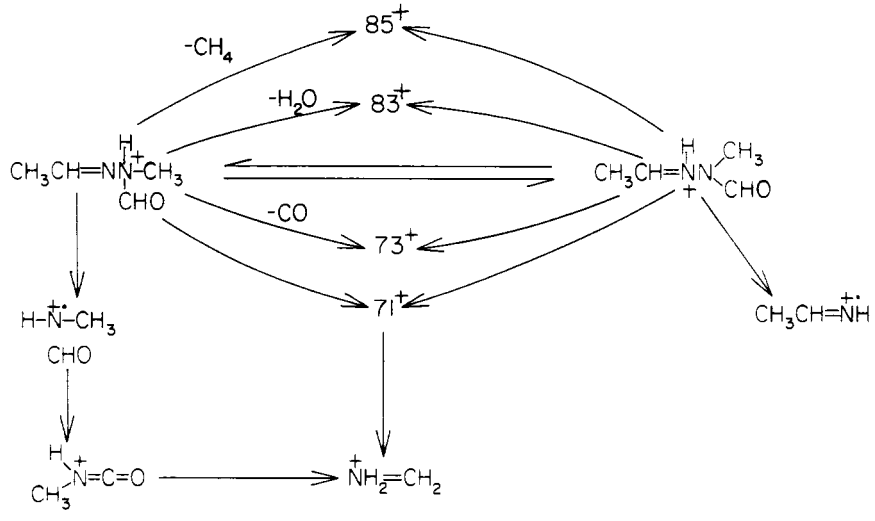


FIGURE 9. Comparison of ms/ms (daughter) spectra of authentic gyromitrin and mushroom tissue sample. (From reference 54)



SCHEME 3

lished. The time profiles of the ms/ms spectra were important in recognizing trewiasine in these experiments.

An alternative to examining daughter or parent spectra is to use neutral loss spectra to characterize functional groups (56). Mass spectra are often interpreted by considering the small, neutral, fragments lost from the molecular ion. For example, because protonated phenols readily lose water in the ci mass spectrum, such an observation is diagnostic of a phenol, although not exclusive to phenols. By scanning a mixture of protonated molecules and rejecting all but those which lose 18 mass units (H_2O), one is, in effect, screening for phenols. Such a process can be effected by the technique of covariant scanning of the two mass analyzers in an ms/ms instrument, while maintaining a constant mass difference between them. The test is, of course, subject to false positives, but these can be minimized by applying a sequential test, *e.g.*, one based on the fact that in negative ci, phenols lose CO (28 mass units). Individual phenols are characterized specifically by their daughter spectra, once their masses have been established by neutral loss scans. Neutral loss scans have found application in examining very complex coal-derived mixtures, and they should prove useful in the natural products area, too (57).

QUANTITATION.—Relatively little effort has been spent on quantitation of natural products by ms/ms. The procedures, and even the software, are borrowed from gc/ms. Both standard addition and internal standards are used successfully. Calibration curves may be made using multiple reaction monitoring or full scans. An example of a calibration curve is given in Figure 10, which illustrates the single reaction monitoring of cocaine (304^+ to 182^+) in MeOH solution. The curve is linear over two to three or-

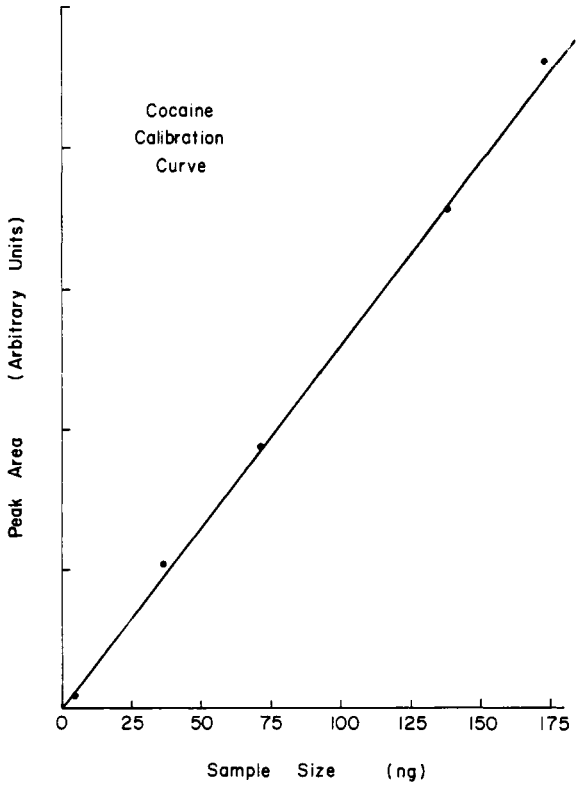


FIGURE 10. Calibration curve for quantitation of cocaine by monitoring the reaction 304^+ to 182^+. (From reference 27)

ders of magnitude, and the useful lower limit is *ca.* 1 μg, where the error is estimated as 30% (27). More recently this sort of precision has been achieved at the ng level (58). When making quantitative measurements without internal standards, variations in the matrix of the analyte need to be minimized (27). The volume of the solvent as well as the rate of heating should be constant to make the quantitative measurements as reproducible as possible.

INSTRUMENTATION.—To utilize the powerful capabilities of ms/ms, instrumentation of some complexity is necessary. Mikes is a high energy form of ms/ms in which magnetic sector analysis precedes electrostatic sector analysis, an arrangement known as reversed geometry. This was the first device used for direct mixture analysis (59).

Low energy ms/ms can be done on quadrupole instruments where collision energies are typically 5-50 eV (10) and two independent mass filters are used for mass analysis (4). An rf-only quadrupole that can be pressurized with a collision gas is used as a focusing device, and the result is an array of three quadrupoles of which just two are used as mass analyzers. The double quadrupole instrument is similar, except that the collision occurs in the interquadrupole region (60). Hybrid instruments employ both sectors and quadrupoles to combine the high resolution of sector mass spectrometers and the versatility of quadrupoles (10). For this type of arrangement, a series of deceleration lenses are necessary for the transition between the high energy sector region and the low energy quadrupole region.

Because ms/ms exceeds even gc/ms in the speed with which data can be accumulated, automation of the mass spectrometer and data processing is highly desirable if full advantage is to be taken of the methodology. This allows matching of library ms/ms spectra to be done using the routines already developed for gc/ms spectral comparisons. It also allows more complex two-dimensional experiments to be performed than are accessible using simple analog scans.

NEW DIRECTIONS.—This review has covered a variety of experiments just beginning to have an impact in natural products chemistry. We can expect to see much more extensive utilization of these capabilities in the near future. The very high sensitivity of ms/ms has not yet been fully exploited in the natural products area. The potential of the method to contribute to biosynthetic studies awaits utilization. One can also look for additional applications in which secondary metabolite distributions are measured as a function of geographical, temporal, and species variations. The high productivity of the method allows multiple samples to be screened for new or targeted compounds with minimum investment in sample preparation.

One can also look for new types of measurements to be introduced. The possibility exists that one could map tissue on a microscopic scale for particular compounds using a probe beam in a desorption ionization experiment. The direct examination of thin layer chromatograms using ion and laser beams, a procedure already demonstrated, should allow established procedures of alkaloid isolation to be used to maximum advantage in conjunction with the powerful new instrumental capabilities.

While the available capabilities of multiple analyzer ms are brought into the main stream of natural products chemistry, new developments in instrumentation and methodology continue. For example, the capabilities of ms/ms are being extended by obtaining spectra on ions m_1^+ of particular internal energies. If a series of ms/ms spectra is recorded as a function of the energy deposited in m_1^+, one obtains information that simulates the breakdown curve (5). The plot in Figure 11 is a much more complete description of the fragmentation of an ion than is a single ms/ms spectrum taken under particular conditions. In a number of cases, isomeric ions can be distinguished via their breakdown curves, where such distinction is difficult by ms/ms alone

(5). Applications of these capabilities to more complex systems are awaited, but this higher dimensional experiment is easily implemented by quadrupole ms/ms spectrometers where the collision energy itself is varied to set the internal energy deposited and by sector instruments where this is achieved by varying the scattering angle (47). Even without such extended capabilities, tandem mass spectrometry appears, from the perspective of a natural products chemist (RGC) who later adopted mass spectrometry, to be particularly suited to natural product applications.

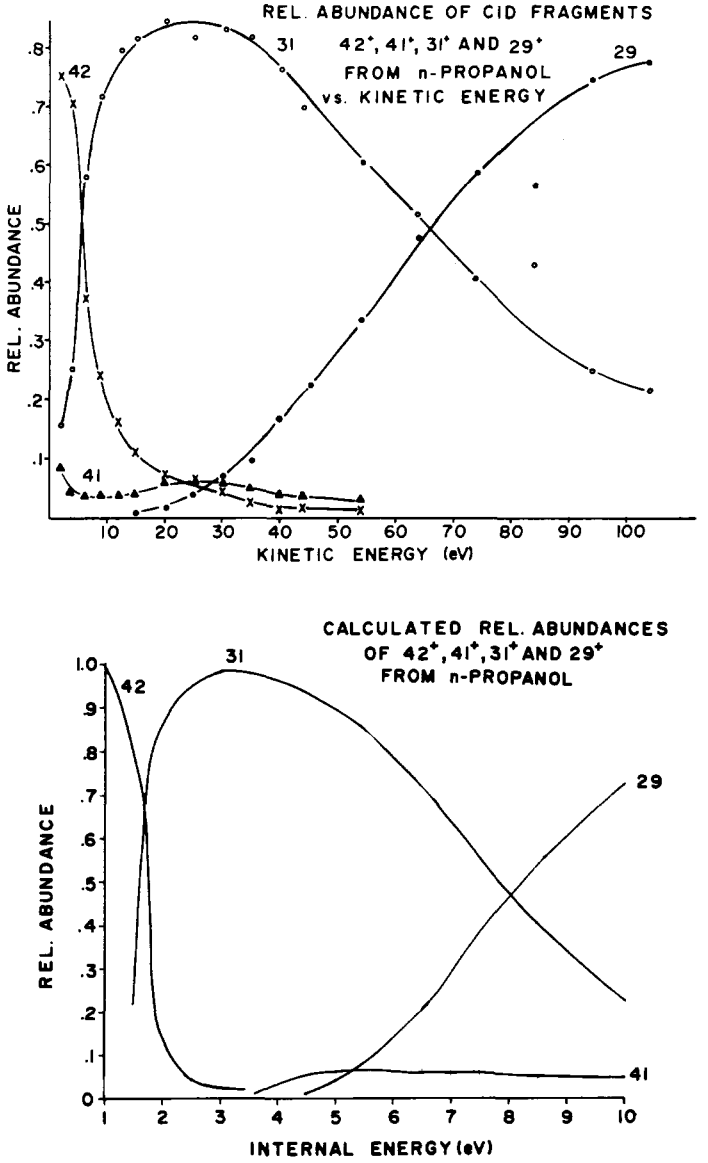


FIGURE 11. Experimental and calculated energy breakdown curves of *n*-propanol. (From reference 5)

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GLOSSARY

Tandem mass spectrometry (ms/ms): family of techniques in which two stages of mass spectrometry are used to separate and to characterize individual components in mixtures.

Collision-induced dissociation (cid): the fragmentation of a parent ion due to collision of the ion with a target gas (also referred to as collision activated dissociation).

Mass-analyzed ion kinetic energy spectrometry (mikes): an ms/ms technique using a high-energy double-focusing mass spectrometer that has the magnetic sector preceeding the electric sector (61).

Desorption ionization: production of ions directly from the condensed phase by energy input; includes sims, fab, ld, etc.

Secondary ion mass spectrometry (sims): A surface technique for analyzing nonvolatile substances by desorption of secondary ions as a result of bombardment by a primary ion beam.

Fast atom bombardment mass spectrometry (fabms): a method of bombarding a solid or liquid surface with fast atoms, resulting in ionic species being desorbed.

Metastable ion dissociation: spontaneous dissociation of a parent ion to a daughter ion after exiting the ion source (55).

Charge-stripping reaction: the loss of electrons by the parent ion upon collision with a target gas.

Charge inversion reaction: the change of polarity of the parent ion due to the loss of electrons upon collision with the target gas.

Isobars: species with the same integral mass.

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