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Tandem Mass Spectrometry (MS/MS): A Promising New Analytical Technique for Specific Component Determination in Complex Mixtures

FRED W. MCLAFFERTY

Cornell University, Department of Chemistry, Baker Laboratory, Ithaca, New York 14853

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The separation and identification capabilities of the mass spectrometer (MS) have led to many useful applications, such as the separation of isotopes and the identification of elements and molecules. The combination of these capabilities with tandem mass analyzers (MS/MS)[1] appears to provide a mixture analysis technique of unusual specificity, sensitivity, and speed.[1-3] MS/MS is conceptually analogous to the combinations of gas and liquid chromatography with mass spectrometry (GC/MS[4] and LC/MS[5]) in which the sample mixture is separated chromatographically followed by on-line component identification by MS. Such separation/identification systems have well-recognized capabilities for highly selective analyses of trace components in complex mixtures. This has become increasingly critical for such important problems as pollution monitoring, chemical diagnoses using body fluids, insect control with natural pheromones, chemical taxonomy, drug metabolite identification, petroleum characterization, and forensic applications.

The MS/MS analysis of a hypothetical mixture containing components ABC, DEF, etc., is shown schematically in Figure 1. Continuous introduction and ionization of the mixture produces ions such as ABC'^{+}, DEF'^{+}, etc., representative of the components sought. To measure component ABC, MS-I is set so that only ABC'^{+} ions pass into the fragmentation region. Here metastable ion (MI) decompositions,[6] or those induced by collisional activation (CA) with an inert gas,[7] yield fragment ions AB^{+}, BC^{+}, etc., whose m/z and abundance values measured in MS-II constitute a mass spectrum characteristic of the component ABC. Thus quantitative analysis for ABC can be based on $[AB^{+}]$, even in the presence of isomer ACB if the latter does not lead to the formation of AB^{+}. Similarly, MS-I can be set to transmit DEF'^{+}, or primary ions representing other components, allowing their MI or CA mass spectra to be measured by MS-II.

As with both chromatography and mass spectrometry, the concept of MS/MS analysis was demonstrated[8] many years before such applications were extensively pursued; earlier studies have been reviewed.[1-3] Very recently there has been an unusual outpouring of reports concerning promising applications. Particularly noteworthy work has originated from the laboratory of R. G. Cooks of Purdue[2,9-12] as well as those of K. Levsen of Bonn,[13] A. Maquestiau and R. Flammang of Mons,[14] A. L. Burlingame of Berkeley,[15] M. Anbar of the Stan-

Fred W. McLafferty received B. S. (1943) and M.S. (1947) degrees from the University of Nebraska, interrupted by service in the U.S. Army/Infantry in Europe. After receiving his Ph.D. degree from Cornell, he was in charge of mass spectrometry and gas chromatography at the Dow Chemical Company and then director of Dow's Eastern Research laboratory for basic research. He became Professor of Chemistry at Purdue University in 1964 and at Cornell in 1968. His research interests include mechanisms of mass spectral decompositions, kinetics of unimolecular ion reactions, LC/MS, MS/MS, mass spectra of nonvolatile compounds, and computer identification of unknown mass spectra.

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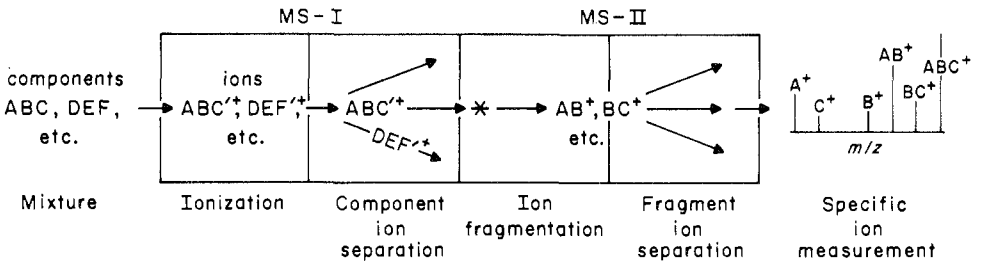

Figure 1. MS/MS analysis of complex mixtures.

ford Research Institute,[16] J. R. Knowles of Harvard,[17] C. N. McEwen of Du Pont,[18] Yost and Enke of Michigan State,[19] and D. F. Hunt of Virginia.[20] This Account of MS/MS covers operating principles, basic capabilities and limitations, instrumentation, examples of applications, and areas of promise for the future.

Operating Principles

In most studies to date the required tandem mass analysis has been achieved with the magnetic and electrostatic analyzers of a "reversed-geometry" double-focusing mass spectrometer. Such instruments are available commercially[21] or from the reversal of the conventional Nier–Johnson geometry.[22] Fragmentation of the m_1^+ primary ions leaving MS-I produces m_2^+ ions whose kinetic energy is directly related to the mass fraction m_2/m_1; thus an electrostatic analyzer can serve as MS-II, with voltage scanning producing a linear mass scale.

Primary Ion Fragmentation. Metastable primary ions of the required half-life ($\sim 10^{-5}$ s) can decompose between MS-I and MS-II, producing a "metastable ion (MI)" mass spectrum of secondary ions. In particular cases these very low energy decompositions can have unique analytical utility, such as sensitivity to isomeric differences[23] or molecular geometry.[6] However, usually there are very few (or no) peaks in such MI spectra, and their abundances are <0.1% of that of the precursor ion.

A secondary mass spectrum of many more peaks of greater abundance is obtained by collisional activation (CA) of the separated component ions.[7] The primary ions leaving MS-I have several kilovolt translational energy from the ion source acceleration; a small portion of this (1–25 eV) can be converted into internal energy by a "near-miss" collision with a molecule or atom, such as helium (a few centimeters path length at 10^{-4} torr is sufficient). The resulting ion decompositions are similar to those found in normal electron-ionization (EI) mass spectra and can be interpreted by similar rules for structural elucidation. A further advantage is that the product ions from higher energy decompositions (those not found in the MI spectrum) show relative abundances that are virtually independent (±5%) of the ion's internal energy, and are thus characteristic of the ion's structure,[7] independent of its mode of formation. The CA energy deposition function is predictable from Massey's adiabatic criterion;[24] increasing the primary ion kinetic energy increases the abundance of fragment ions from high-energy CA decompositions and thus also increases the sensitivity for CA spectra.

MI and, especially, CA spectra obtained using such an energy analyzer can exhibit broadened peaks. Part of the excess reaction energy of the decomposition can appear as translational energy of the products, which will increase or decrease the overall kinetic energy of the product ion according to the spatial orientation of its precursor at decomposition. Although the amount of energy released can be used to characterize the precursor ion,[6] the resulting peak broadening can seriously limit the resolution of the many peaks in CA spectra. Increasing the ion accelerating voltage decreases the relative amount of ion translational energy arising from such energy release and thus improves the resolution.[6,22,25] Unit resolution CA spectra can be obtained by the linked scan technique,[26,27] but this gives poor resolution of the precursor ions[28] so that anomalous peaks from other precursor fragmentations often interfere.

Basic Capabilities and Limitations

The capital, maintenance, and personnel costs of a double-focusing instrument for MS/MS limit its analytical applicability to problems poorly amenable to GC/MS or LC/MS, although development of the tandem quadrupole[19,20] described below promises to reverse this situation. Much larger and more polar molecules can be analyzed by MS/MS (and LC/MS) than by GC/MS because of the much lower sample vapor pressure requirements ($\sim 10^{-4}$ torr) of MS. MS is a virtually instantaneous ($\sim 10^{-5}$ s) separation method, while chromatographs require minutes to hours for separation at high resolution; this greatly enhances the applicability of MS/MS to routine analyses and on-line stream monitoring. MS separating capabilities, such as for stable isotopes, generally complement those of

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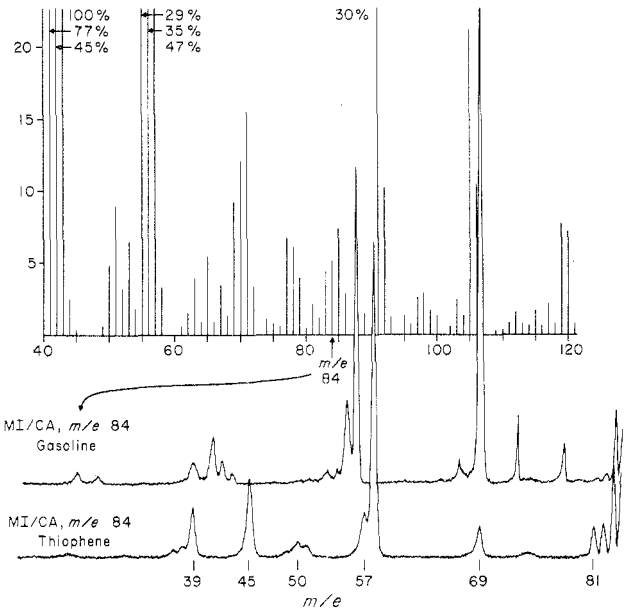

Figure 2. Top: EI mass spectrum of gasoline (Exxon regular). Middle: MI/CA mass spectrum of its m/z 84 ions. Bottom: MI/CA spectrum of the EI m/z 84 ions of thiophene.

chromatography; the hundreds of peaks separable by MS-I are comparable to the number in GC and LC chromatograms while high-resolution MS separation[29-31] increases this possible number by more than two orders of magnitude. An advantageous capability common to GC/MS, LC/MS, and MS/MS is selected ion monitoring[1-5] for the specific analysis of a targeted compound; compounds of a specific type can be detected by monitoring their characteristic ion masses or mass differences. The subpicogram sensitivities reported in numerous GC/MS applications have recently been achieved[19b,20] using MS/MS. New techniques for collisional activation give secondary ion yields of 10–75%,[19,20,30] approaching carrier gas separation efficiencies in GC/MS. The collision chambers employed in most CA studies to date, however, have efficiencies of <1%.

Component Ionization of MS-I. A major use of GC/MS has been the analysis of complex mixtures for which the identities of all components are desired. For this a serious limitation of MS/MS is the difficulty of ionizing a mixture to give a peak for every component without giving more than one peak for many components, even with "soft" ionization methods such as chemical and field ionization[1-4,13,32] and field desorption.[27] On the other hand, this variability of response of mixture components to the wide variety of ionization methods now available means that particular components can often be ionized with high selectivity, an obvious advantage for *specific* MS/MS analysis of particular components. For example, efficient chemical ionization (CI) of a component by protonation requires a reagent gas lower in proton affinity. Components with electronegative substituents such as nitro or halogen give high yields of negative ions by CI.[32] Compounds of low ionization potential, such as benzopyrene, can be selectively ionized with low-energy electrons. Fractional vaporization of a mixture sample with normal direct probe introduction in EI or CI produces a time dependence in component ionization,[4] as does programming of the emitter temperature in field desorption.[27]

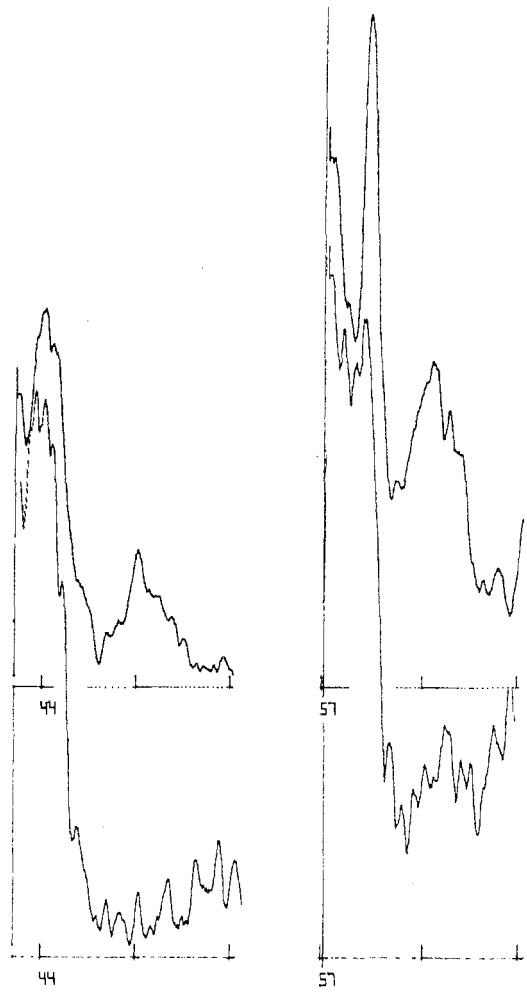

Figure 3. Partial MI/CA spectra (computer average of 16 scans) of EI m/z 84 ions from samples of gasoline (lower trace) and 50 ppm thiophene in gasoline. The sharp peak at m/z 57.5 is apparently due to ions formed by metastable ion decompositions occurring in the first field-free drift region.

Analytical Applications

Hydrocarbons. As indicated in the first investigations utilizing MS/MS,[8] this technique appears promising for complex hydrocarbon mixtures.[1] The EI mass spectrum of gasoline shows (Figure 2) peaks at most masses. Using a reverse geometry instrument, the mass 84 peak can be separated in MS-I and subjected to collisional activation and its CA mass spectrum measured in MS-II. This resembles the normal EI mass spectrum of C_6H_{12} molecules such as hexenes or cyclohexane, but is substantially different from the CA spectrum of the mass 84 molecular ion of thiophene (Figure 2). In fact, the abundant peaks in the latter at m/z 45 (CHS^+) and 58 ($C_2H_2S^+$) are not present in the $C_6H_{12}^+$ spectrum, as this would require formation of fragments such as $C_3H_9^+$ and $C_4H_{10}^+$, respectively. These portions of the CA mass spectrum of mass 84 from gasoline measured at high sensitivity are compared

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(30) Todd, P. J.; McGilvery, D. C.; Baldwin, M. A.; Wendel, G. J.; Wixom, M. R.; McLafferty, F. W. Ref 12, Paper WAMP2.
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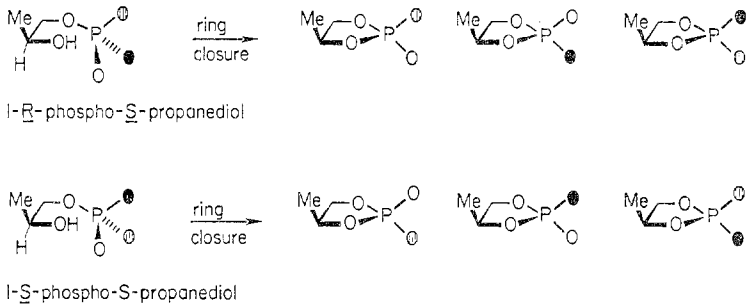
(32) Hunt, D. F.; Stafford, G. C. Jr.; Crow, F. W.; Russell, J. W. *Anal. Chem.*, **1976**, *48*, 2098.

Scheme I



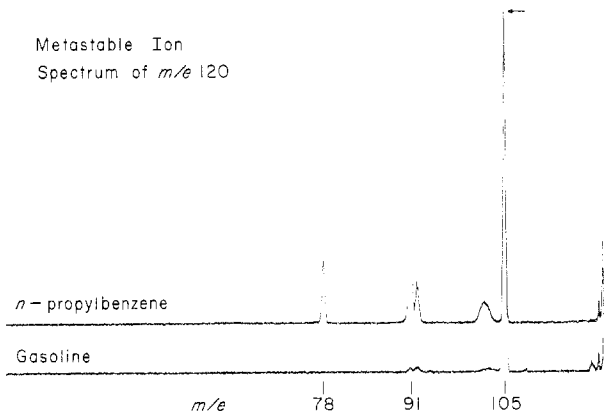


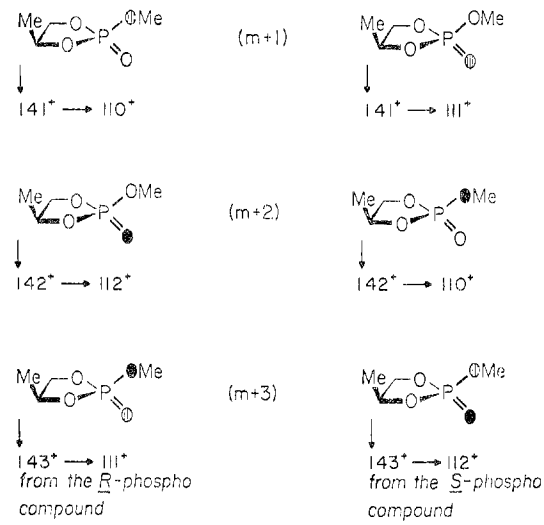
Figure 4. MI spectra of the EI m/z 120 ions from gasoline and *n*-propylbenzene.

(Figure 3) to the same data from a gasoline sample to which 50 ppm of thiophene have been added. Detection sensitivities below this level should thus be possible with continuous analysis.

These isobaric ions, $C_6H_{12}^+$ (m/z 84.094) and $C_4H_4S^+$(m/z 84.003), could have been separated by high-resolution MS, making analysis with appropriate instrumentation possible. This is not true, however, for the C_9H_{12} isomers such as trimethylbenzenes, isopropylbenzene, and ethylmethylbenzenes which yield the large peak at m/z 120 in the gasoline EI spectrum (Figure 2). The MI spectrum of this separated mass 120 peak is shown in Figure 4. The corresponding MI spectrum of the m/z 120 molecular ion from *n*-propylbenzene exhibits a large mass 91 peak ($C_7H_7^+$) arising from benzylic cleavage, while this cleavage for the other C_9H_{12} isomers gives little m/z 91. This makes possible MS/MS detection of ~500 ppm of *n*-propylbenzene in the presence of much higher concentrations of several isomeric compounds. Detection of *n*-propylbenzene based on the m/z 91 peak in the normal mass spectrum (Figure 2) would be impossible because here this peak arises mainly from the C_7H_8 and C_8H_{10} components.

DNA Pyrolysis. Using MS/MS Levsen and Schulten[13] identified directly six major volatile components of the complex mixture produced by pyrolysis of deoxyribonucleic acid from herring. Molecular ions were enhanced in the primary mass spectrum by using low-voltage (14 eV) electron ionization, and component identifications were confirmed by comparison of unknown and reference CA spectra. Schoen, Cooks, and Wiebers[9] pyrolyzed salmon sperm DNA directly from the MS sample probe into a CI source. CA spectra of separated m/z 126 and 150 primary ions showed the presence of the bases 5-methylcytosine, which originates

Scheme II



from the rare deoxynucleoside 5-methyldeoxycytidine, and 1-methyladenine, which was distinguished from the 2-methyl and N^6-methyl isomers.

Crude Natural Product Mixtures. Several years ago Smith, Djerassi, and colleagues[33] showed that simple steroid mixtures could be separated and identified in a reversed-geometry double-focusing mass spectrometer using MI spectra of the separated molecular ions. Maquestiau and co-workers[14] report that complex marine sterol mixtures can be analyzed efficiently and rapidly, using MI and CA spectra of EI molecular ions. Cooks and his students have described diverse natural product analyses by MS/MS which involve a minimum of workup for samples such as plant materials and urine. A variety of ionization conditions (negative as well as positive CI) and sample introduction procedures can be used to optimize sensitivity and selectivity.[10] The distribution of cocaine and cinnamoylcocaine in whole coca plant tissues (leaves, stems, and berries) can be mapped on a scale of 1 mm^3 with no sample preparation.[11]

Chirality of Phosphate Monoesters. Knowles and his co-workers have developed an ingenious technique for determining the chirality of phosphate monoesters which can be used to elucidate mechanisms of key biological reactions such as phosphoryl transfer.[17] The starting phosphate is synthesized with the isotopes ^{16}O, ^{17}O, and ^{18}O in a known absolute configuration. Ring closure (Scheme I) of the phosphate can displace any one of the three oxygen atoms, producing a mixture

(33) Smith, D. H.; Djerassi, C.; Maurer, K. H.; Rapp, U . *J. Am. Chem. Soc.*, 1974, *96*, 3482.

Table I
Proportion of *R* Configuration Indicated by the Six Possible Isotopic Isomers for Partially Racemized (*R*)-Phospho Sample

precursor mass	% *R* using diastereomeric set	
	syn	anti
141	58[a]	48[a]
142	52	52
143	54	58
	av[a] 54	av[a] 54

[a] Accuracy using precursor *m/z* 141 is less because of interfering ions, and these values were given only half weight in the average.

with three isotopic combinations of the remaining pair. Methylation yields six possible isomers but these can be separated into the "syn" and "anti" diastereomeric sets of three by liquid chromatography (the *R* and *S* "syn" sets are shown in Scheme II). Ring opening yields products for which the mass spectrum of the unlabeled derivative produces a prominent *m/z* 140 ion of the structure $(CH_3O)_3P{=}O^{+}\cdot$. The MI spectrum of this ion shows a major peak for CH_2O (mass 30) loss, allowing isotopic labeling in CH_3O- and P=O to be distinguished. The mixture of Scheme II produces ions of *m/z* 141 (^{16}O, ^{17}O), 142 (^{16}O, ^{18}O), and 143 (^{17}O, ^{18}O). By MS/MS the *m/z* 141^+ ion can be separated and its MI spectrum determined; loss of mass 31 shows the presence of $^{17}OCH_3$ and the *R* chirality of phosphorus, while the *S* isomer can be measured using the peak formed by loss of 30. The analysis can be confirmed using the decompositions of the separated 142^+ and 143^+ peaks also, and, in fact, remeasured three more times with the other diastereomeric mixture. Table I shows the results of such an analysis on an *R* sample in which the cyclization conditions had caused almost complete racemization.

Peptide Sequencing. An earlier Cornell report[34] described determination of amino acid sequences in oligopeptide mixtures for components in 5–10% concentration and 10–20-nmol quantities. MI spectra of separated fragment ions (EI and CI) contained at least partial sequence information, with additional information indicated from CA spectra. Although their EI spectra are usually closely similar, leucine and isoleucine can often be distinguished using such secondary MI or CA spectra.[34b] Very recently Hunt and co-workers[20] have reported sequencing 23 oligopeptides in a mixture from elastase digestion of glucagon, a 29-residue peptide, entirely by MS/MS. The mixture is N-acylated with 1:1 CH_3CO-/CD_3CO- followed by permethylation, and subjected to positive ion chemical ionization. Any $(M + H)^+$ or N-terminal fragment ions thus give a pair of peaks separated by 3 mass units. This *pair* of peaks is separated by MS-I using degraded resolution; the resulting CA spectrum then has similar pairs of fragment ions formed by peptide chain cleavage retaining the N terminus which delineate the sequence. A potential 100–1000-fold increase in sensitivity is indicated for N- or C-terminal primary fragment ions using electron capture negative ion CI[32] with *N*-pentafluorobenzoyl or pentafluorobenzyl ester derivatives, respectively.

(34) (a) Wipf, H.-K.; Irving, P.; McCamish, M.; Venkataraghavan, R.; McLafferty, F. W. *J. Am. Chem. Soc.*, **1973**, *95*, 3369. (b) Levsen, K.; Wipf, H.-K.; McLafferty, F. W., *Org. Mass Spectrom.*, **1974**, *8*, 117.

Improved Instrumentation

Tandem Quadrupole. Yost and Enke[19] have recently reported that a multiple quadrupole instrument, constructed by McGilvery and Morrison for photodissociation studies,[35] has very attractive advantages for MS/MS. Separated primary ions leave the MS-I quadrupole to undergo collisions in a low-pressure ($\sim 10^{-4}$ torr) region in which the ions are confined by an RF-only quadruple (no mass separation).[36] This transmits the CA product ions with high efficiency to a third quadrupole for mass analysis of the resulting CA spectrum. Relatively high collision efficiencies (≥25%) are observed[19,20] because product ions resulting from direct collisions can be transmitted (for the high-energy ions described above the activation is caused by "near-miss" collisions). Although ion collision energies are much smaller (tens of volts) in the tandem quadrupole instrument, such direct collisions can convert a relatively high proportion of the ion translational energy into internal energy (favored by a high molecular weight collision gas),[37] so that the resulting CA spectra are surprisingly similar to those produced by the grazing collisions of high-energy ions (as expected from the quasiequilibrium theory).[24] A main difference is that ion–molecule reaction products are sometimes observed in the tandem quadrupole, such as CCl^+ with Ar producing Ar^+, CAr^+, and $ClAr^+$.[19] The tandem quadrupole is particularly promising as a routine analytical instrument for reasons that the quadrupole is favored for GC/MS, such as the ease with which it can be computer controlled and its lower cost on mass production.

Improved Resolution for MS-II. A further striking advantage of the tandem quadrupole is that its CA spectra show unit mass resolution. For reversed-geometry instruments the poor resolution (vide supra) using the MS-II energy analyzer can be improved by raising the precursor ion kinetic energy. It is better not to do this by increasing the ion source accelerating potential, which reduces the maximum *m/z* value for ion separation by the MS-I magnet. Boerboom and co-workers[25] achieve ≤40-kV ion kinetic energies in a new instrument using post-collision acceleration and a magnetic analyzer for MS-II. The new Cornell instrument (Figure 5)[29,30] has the capability of 20-kV acceleration between MS-I and the collision region, giving total ion kinetic energies of ≤30 kV, providing improved resolution when only an electrostatic analyzer is used as MS-II. A 23-kG magnet is being added to this instrument to make MS-II double-focusing; as with the spark-source mass spectrometers, this brings ions of different energies (±50 V) but of the same mass to a common focal point. MS-II of Figure 5 should have a resolving power of >5000.

High-Resolution MS-I. Capillary column GC is revolutionizing GC/MS because of its greatly increased

(35) McGilvery, D. C.; Morrison, J. D. *Int. J. Mass Spectrom. Ion Phys.*, **1978**, *28*, 81–92.

(36) A very recent report describes a tandem system of only two quadrupoles, between which is injected the collision gas; the performance appears to be very promising: Glish, G. L.; Unger, S. E.; Schoen, A. E.; Zakett, D.; Ridley, T. Y.; Cameron, D.; Sigsby, M. L. Kruger, T. L.; Cooks, R. G. 178th National Meeting of the American Chemical Society, Washington, DC, Paper ANAL 95.

(37) Leventhal, J. J.; Friedman, L. *J. Chem. Phys.*, **1968**, *49*, 1974. In fact, the instrument used by these authors (*Ibid.*, **1969**, *50*, 2928) to determine threshold values for ionic reactions from center-of-mass collision energies should serve equally well for such MS/MS experiments.

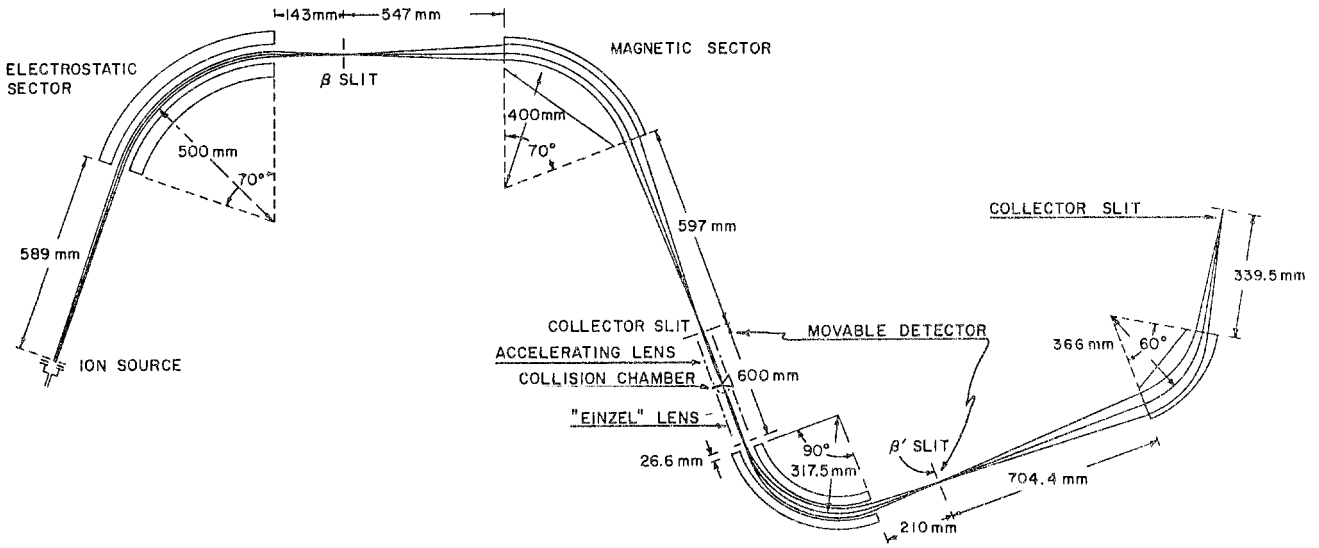


Figure 5.

separation capabilities. Improved separation should be similarly useful for MS/MS; the new Cornell instrument (Figure 5) has a usable resolving power of 50 000 for separation of mixture component ions in MS-I. Thus 50 separable peaks are possible between m/z 1000 and 1001.[30] Such high-resolution MS-I capabilities will also be available on several "triple-analyzer" instruments now under construction through the addition of a collision chamber and electrostatic analyzer to a double-focusing MS.[31] Initial applications which appear promising include parts per trillion level determination of tetrachlorodibenzodioxin with less sample workup, direct analysis of complex biological samples such as blood or urine for specific compounds such as digitoxin, and polypeptide sequencing (vide supra) involving ion source degradation of the polypeptide into a complex oligopeptide mixture to be separated in MS-I with component sequencing in MS-II. Note that tandem double-focusing mass spectrometers are already used for ultratrace analysis; for nuclear test monitoring of nuclidic elements abundance sensitivities of $1/10^{12}$ are achieved routinely,[38] while a related instrument can detect three atoms of ^{14}C in 10^{16} atoms of ^{12}C.[39] In the new Cornell MS/MS a molecular beam collision region at the focal point of the ion lens system makes possible yields of CA product ions of 10%,[30] so that the completed tandem double-focusing instrument could be uniquely sensitive as well as selective for specific component determination.

GC/MS/MS and LC/MS/MS. There appears to be a never-ending increase in the demand for analyses of higher sensitivity and selectivity. The combination of GC and MS has helped meet this demand by adding another dimension of specificity over analyses by either individual technique. MS/MS is a much more specific detector than MS alone; thus adding MS/MS to GC or LC (or another MS) is an obvious way to increase selectivity further. Even crude separations, such as fractional vaporization from the MS direct probe, can aid the selectivity of MS/MS. Note that an MS/MS detector could monitor for all separated components giving a fragment ion of a characteristic structure, selecting the corresponding m/z value with MS-I and checking for the CA spectrum characteristic of that isomer with MS-II.

The added MS also offers a way to overcome a major drawback with the direct solution introduction interface for LC/MS.[5] In this method ~0.05 mL/min of the LC effluent is continuously introduced into a CI source, with the solvent acting as the ionizing reagent gas.[40] Often the fragment ion abundance from chemical ionization is not sufficient to give useful structural information, providing only an abundant $(M + H)^+$ or other ion with molecular weight information. However, the second MS can provide the CA mass spectrum of this molecular-type ion, so that such an LC/MS/MS instrument could yield both high selectivity for component separation and extensive information for component identification.

Concluding Remarks

A chromatogram and a mass spectrum can each have hundreds of separated peaks; the multidimensional combination of pairs of these as MS/MS, as well as GC/MS and LC/MS, can provide unusually high selectivity, which with the sensitivity of MS (capable of counting individual ions) produces a uniquely powerful analytical tool. New MS/MS applications and instrument improvements are being reported at a surprising rate, reminiscent of the pace reached in GC/MS developments a decade ago. Several instrument makers appear interested in bringing out commercial MS/MS instruments designed for service analyses. The computer-controlled tandem quadrupole is particularly promising for this, and could be competitive in price with GC/MS. High-resolution MS/MS should be uniquely applicable to key problems requiring ultratrace analysis with a minimum of sample workup, although here GC/MS/MS and LC/MS/MS should also prove to be of real value.

A very extensive list of my research collaborators over many years deserves the bulk of the credit for the research reported here. Thomas Bryce did the first MS/MS studies in our laboratory at Purdue, concurrent with Bill Haddon's initial work on collisional activation. Our early MS/MS studies on peptide

(38) White, F. A.; Forman, L., *Rev. Sci. Instrum.*, **1967**, *38*, 335.
(39) Bennett, C. L. *Am. Scientist*, **1979**, *67*, 450.

(40) Commercial LC/MS instruments using direct solution introduction are available from Hewlett-Packard, Palo Alto, CA, and Ribermag, Paris.

mixtures were done by Phil Irving, Hans-Kasper Wipf, Malcolm McCamish, Babu Venkataraghavan, and Karsten Levsen. I am particularly indebted to Tim Wachs and Paul Bente for reversing the geometry and computerizing the Hitachi RMU-7 used in the bulk of our MS/MS research in the last decade. The design of the new tandem double-focusing MS was done largely by Peter Todd with the construction by him, Mike Baldwin, Don McGilvery, Mike Barbalas, Greg Wendel, and Mike Wixom, and with important contributions from Henk Boerboom, Peter Derrick, and Richard Porter. Frank Bockhoff performed the extensive recent MS/MS studies including those on the gasoline mixture and phosphate chirality. Myung Kim greatly improved our basic understanding of the collision process. Finally, none of this research would have been possible without the generous financial support of the National Institutes of Health and the Army Research Office, Durham.

Intramolecular Iminium Ion–Sulfide Charge-Transfer Association: A Recurring Theme in the Study of Thiaspirane Alkaloids

ROBERT T. LALONDE

Department of Chemistry, State University of New York, College of Environmental Science and Forestry, Syracuse, New York 13210

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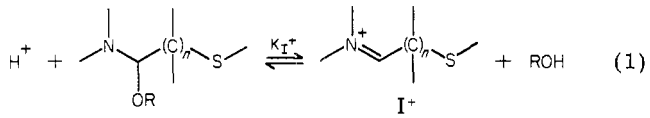
The aquatic macrophyte *Nuphar luteum*, commonly known as the yellow pond lily or spatterdock, produces piperidine and quinolizidine alkaloids having a regular sesquiterpenic carbon skeleton.[1] The quinolizidine structure is also incorporated, along with a sulfur atom, in a C_{30} dimer alkaloid, referred to as a thiaspirane. Through configurational change at C-7 and C-7′, four stereoisomeric types are possible (see Figure 1). Indeed three, **1**–**3**, of the four types have been isolated and the fourth, **4**, has been prepared from **1**.[2] The three natural thiaspiranes occur as diamines (R_1, R_2, R_3, R_4 = H), monohemiaminals (R_1, R_2 = H, OH; R_3 = R_4 = H or R_1 = R_2 = H; R_3, R_4 = H, OH) and bishemiaminals (R_1, R_2 = R_3, R_4 = H, OH). However in comparison to the diamines, the thiaspirane hemiaminals proved the more interesting since these possessed exceptional properties attributed to the intramolecular charge-transfer association of sulfur with iminium ion, the latter being in equilibrium with the hemiaminal as shown in eq 1.



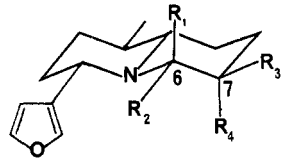
I-OR (1)

Thus this Account is concerned with α- (I^+, n = 1) and β-thioiminium ions (I^+, n = 2), the corresponding thiohemiaminals (I-OR, R = H), and the manifestation of the charge-transfer association whose character is supported by additional observations disclosed herein. However for the most part the observations discussed here are those made in the course of structure studies carried out in my laboratory and others over the course of recent years.

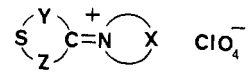
Robert T. LaLonde received his B.A. degree from St. John's University, MN, in 1953 and the Ph.D. from the University of Colorado, Boulder, CO, in 1957. After being employed by the Jet Propulsion Laboratories in Pasadena, CA, he engaged in postdoctoral research at the University of Illinois, Urbana, and joined the SUNY College of Environmental Science and Forestry in 1959. He is now Professor of Chemistry. Among his current research interests are natural products of significance to aquatic ecology.

Optical Properties Consistent with an Intramolecular Charge-Transfer Association

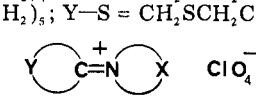
An early indication of sulfur–iminium ion interaction came from the UV spectra of hemiaminals. Thus spectra of thiohemiaminals such as **3** (R_1 = R_2 = H; R_3, R_4 = H, OH), **3** (R_1, R_2 = R_3, R_4 = H, OH) and the synthesized epimeric pair **5** and **6** in neutral 95% eth-



5, R_1, R_2 = H, OH; R_3 = SCH_3; R_4 = CH_3
6, R_1, R_2 = H, OH; R_3 = CH_3; R_4 = SCH_3
7, R_1 = R_2 = H; R_3 = SCH_3; R_4 = CH_3
8, R_1 = R_2 = H; R_3 = CH_3; R_4 = SCH_3
9, R_1, R_2 = H, OH; R_3 = CH_3; R_4 = OH
17, R_1 = R_2 = R_3 = H; R_4 = CH_3
18, R_1 = H; R_2 = D; R_3 = SCH_3; R_4 = CH_3
19, R_1 = D; R_2 = H; R_3 = CH_3; R_4 = SCH_3
21, R_1 = D; R_2 = H; R_3 = OH; R_4 = CH_3
22, R_1 = H; R_2 = D; R_3 = OH; R_4 = CH_3
23, R_1, R_2 = H, OH; R_3 = p-$SC_6H_4CH_3$; R_4 = CH_3
24, R_1 = D; R_2 = H; R_3 = p-$SC_6H_4CH_3$; R_4 = CH_3
25, R_1 = H; R_2 = D; R_3 = p-$SC_6H_4CH_3$; R_4 = CH_3



10, X = $(CH_2)_4$; Y = CH_2; Z = $(CH_2)_2$
11, X = $(CH_2)_5$; Y = CH_2; Z = $(CH_2)_2$
12, X = $(CH_2)_4$; Y = Z = $(CH_2)_2$
13, X = $(CH_2)_4$; Y—S = $CH_2SCH_2CH_3$; Z = CH_3
14, X = $(CH_2)_5$; Y—S = $CH_2SCH_2CH_3$; Z = CH_3



15, X = $(CH_2)_4$; Y = $(CH_2)_5$
16, X = $(CH_2)_5$; Y = $(CH_2)_5$

anol were transparent beyond 250 nm. But in acidic

(1) J. T. Wróbel, *Alkaloids*, **16**, 181 (1977).
(2) R. T. LaLonde and C. F. Wong, *Can. J. Chem.*, **56**, 56 (1978).

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