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CURRENT TOPICS IN ORGANIC MASS SPECTROMETRY

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Abstract - Although mass spectrometry is accepted as a key analytical tool for many molecular problems, the level of world-wide research activity in this field promises that these capabilities will continue to improve rapidly. Active research areas which will be discussed are liquid chromatography/mass spectrometry/computer systems applicable to complex mixtures of low volatility compounds, computer interpretation of unknown mass spectra, and collisional activation (CA) mass spectrometry. The latter can be viewed as a "double-resonance" technique, as a CA mass spectrum can be obtained of each peak in a normal mass spectrum. A variety of applications of CA/MS to ion and molecular structure determination and to the analysis of complex mixtures will be presented.

INTRODUCTION

Mass spectrometry (MS) is now widely used for molecular analysis in important chemical and biomedical problems. Particularly remarkable growth has been due to special systems combining MS with gas chromatography and the dedicated computer (GC/MS/COM), with applications to such complex mixtures as pollutants, drug metabolites, and insect pheromones. Volatility restrictions of GC separation have led to proposals for the analogous direct coupling of the liquid chromatograph to form LC/MS/COM systems of complementary promise. Another result of the remarkable growth in GC/MS applications is a concommitant exponential rise in the number of unknown spectra to be interpreted. Computer techniques appear to be by far the most promising solution to this problem, and both retrieval and interpretive algorithms for unknown mass spectra will be discussed. The final topic examined is collisional activation (CA) MS, by which a mass spectrum can be obtained for each peak in a normal electron-ionization (EI) mass spectrum. Although CA/MS has been applied to date mainly to mechanistic problems of isomeric ion structures, it appears also to have substantial promise for supplying additional structure information on large molecules and as a separation/identification system (MS/MS) analogous to GC/MS for the analysis of complex mixtures.

LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY

The development of high-performance liquid chromatography (LC) has been handicapped seriously by the need for more versatile, sensitive, and selective detectors. This restriction has been far more serious for LC than for GC, for which MS detection has been so successful, and LC/MS has been proposed as a solution to this problem by several laboratories (Ref. 1-6). Dawkins (Ref. 7) has recently reviewed this subject, so only the major areas and recent developments will be covered here.

A key advantage of modern GC/MS systems is the continuous introduction of the GC effluent into the MS. To accomplish this for LC/MS, the interface must overcome the problem of the tremendous disparity in operating pressure between the LC exit ($\geq$ atmospheric pressure) and the MS ion source ($<10^{-4}$ torr for EI/MS). Tal'roze and coworkers (Ref. 1) succeeded in continuously introducing volatile organic liquids into a conventional EI ion source through a capillary leak system, but flow rates of only $\sim 10^{-6}$ ml/min were possible because of the $<10^{-4}$ torr sample pressure requirements of the ion source. Absorption of polar components causes difficulties which could be alleviated by solvent rinsing, but cross contamination of samples, reproducibility of flow rates, and plugging were problems of the capillary interface system (Ref. 8).

Three general types of LC/MS interfaces have been proposed to surmount this formidable problem: interfaces which employ solvent separation, those which vaporize the total effluent at atmospheric pressure, and those which introduce part of the effluent directly into the low pressure MS ionization source. The most successful system for solvent separation is that

proposed by Scott and coworkers (Ref. 2), now available for Finnigan MS instruments (Ref. 9). In this the LC effluent passes over a moving wire or ribbon which carries a portion of the solution into an evaporation chamber where the solvent is removed, leaving the solute deposited on the wire. The wire continuously moves through a suitable interface directly into the ionization chamber of the mass spectrometer where it is heated to vaporize the solute; either electron impact or chemical ionization spectra can then be obtained in the conventional manner. Transfer efficiencies have been greatly increased in the commercial interface (Ref. 9); flow rates of >1 ml/min for hexane to 0.1 - 0.5 ml/min for water can be tolerated. Useable spectra by either EI or chemical ionization (CI) have been obtained on samples in low nanogram amounts. A novel new approach of Vestal and coworkers (Ref. 10) uses laser vaporization and molecular beam techniques to concentrate the solute; in the present apparatus yields are <1%.

Atmospheric pressure ionization, developed by Horning and coworkers (Ref. 11), is effected with a ^{63}Ni source or a corona discharge, and the resulting ions are sampled through a 25 μm pinhole into a mass spectrometer. Continuous vaporization of an LC effluent in the ionizing region gave a detection limit of 0.5 ng in the effluent solution. The most severe restriction of the API technique is the requirement of sample vaporization at atmospheric pressure; it appears that sample vapor pressure requirements are substantially higher than those of normal direct probe introduction into the mass spectrometer, limiting the number of compounds of insufficient volatility for GC separation which can be run by LC/(API) MS.

An alternative LC/MS interface developed at Cornell utilizes chemical ionization (CI) MS to take advantage of the higher operating pressure (~1 torr) in the CI ion source (Ref. 4-6). In the original instrument this made possible solution flow rates of ~0.01 ml/min, or introduction of ~1% split of the LC effluent; with more efficient cryogenic pumping Arpino (Ref. 12) has achieved a 10% split ratio. Although the sample splitting obviously results in a reduction in sensitivity, the MS thus acts as an essentially "non-destructive" detector; the fractions which cannot be completely identified from the resulting CI mass spectra are available for examination by other techniques. For example, the solution could be dried down on the MS direct probe tip and a conventional EI spectrum obtained. This interface also has the advantage of simplicity; its implementation only requires the addition of a splitter and capillary through the direct sample probe of a CI mass spectrometer, and it is effective with all common LC solvents. This system will also be available on a commercial MS near the date of this publication. A variety of interesting chemical and biomedical applications have been reported using this LC interface on a quadrupole MS by Henion (Ref. 13) as well as Arpino (Ref. 12).

Using a dedicated computer with LC/MS for data acquisition and reduction shows very similar advantages to those already well established for GC/MS/COM systems (Ref. 6). Recently Dawkins (Ref. 14) in our laboratory has applied such a LC/MS/COM system to the automated sequencing of oligopeptides in mixtures, such as those obtained by classical methods of polypeptide degradation. A few nanomoles of the peptide mixture is derivatized (acetylation, methyl esterification) to enhance volatility. This mixture is separated by LC (C-18 reverse phase packing, H_2O/CH_3CN gradient), 1% of the eluent is introduced continuously into the MS, and spectra are scanned repeatedly at a rate of several per LC peak. In mixtures of up to six oligopeptides it was possible to obtain complete sequence information on all components for many, but not all, of the mixtures tried. Interpretation of the results was complicated by numerous by-products formed in the derivatization process, and work is in progress to eliminate this step. The announced availability of micro LC columns using flow rates compatible with total effluent introduction into the MS (100% split ratio) could reduce sequencing sample requirements to the tens of picomoles level.

COMPUTER IDENTIFICATION OF UNKNOWN MASS SPECTRA

A substantial number of laboratories using GC/MS/COM systems produce more than 100 unknown mass spectra per day; obviously identification of these is a formidable task for the human interpreter. The variety of computer systems that have been developed to aid the interpreter have been reviewed by this author in a current issue of this journal (Ref. 15), and so only a summary outlining the Cornell programs will be given here.

Algorithms for the computer identification of mass spectra are now available from the Cornell computer via the TYMNET international computer networking system. Unknown mass spectra can first be compared against a reference file of 41,429 spectra of 32,433 different compounds using the "Probability Based Matching" (PBM) system (Ref. 16). Its demonstrated superiority is due to its "weighting" of mass and abundance data, and to its "reverse search" which is especially valuable for mixture spectra. Unknown spectra for which PBM matches of sufficient reliability cannot be found can then be examined by the "Self-Training Interpretive and Retrieval System" (STIRS) which matches the unknown spectral data in 15 preselected categories against a large reference collection (Ref. 17). The best-matching compounds in each data class indicate possible substructural features; for 179 of these the computer

actually provides a confidence level prediction of the substructure's presence (Ref. 18). A new program can compare the best-matching reference compounds to identify the largest common substructures. PBM or STIRS examination of an unknown spectrum usually requires much less than one minute; a variety of examples will be given.

COLLISIONAL ACTIVATION MASS SPECTROMETRY

"Double resonance" techniques have greatly extended the utility of basic spectroscopic tools for both fundamental research and analytical applications. In mass spectrometry, collisional activation (CA) can be viewed as such a technique, as it allows the measurement of a mass spectrum of each peak in the normal mass spectrum (Ref. 19-21). For example, using a reverse-geometry double-focussing mass spectrometer, a peak in the normal MS can be separated by the magnetic field and caused to dissociate in a special collision chamber; separation of the resulting fragment ions by the electrostatic analyzer yields the CA mass spectrum of that particular ion. Because this spectrum is characteristic of the ion's structure, CA/MS has been applied extensively in fundamental studies of unimolecular ion and ion-molecule reactions and in the separation and identification of mixture components (Ref. 19-24). Each of these applications of CA/MS will be reviewed here, with emphasis on recent results.

Our knowledge of the chemistry of organic ions has been substantially enhanced and clarified by experimental studies of such ions in the gas phase, free from solvent effects. Unfortunately, such experimental information has often been difficult to obtain or ambiguous due to the high reactivity and low concentrations of ions obtainable, and the effect of ion internal energy (temperature) on the ion's reactivity or other measurable characteristics. These disadvantages are obviated in large part by using "collisional activation (CA) spectra" to characterize ion structures (Ref. 19-21). In simplest terms the CA spectrum is a mass spectrum of the ion, and ion structure determination by CA is similar to the structure determination of molecules by normal mass spectrometry. Computer-controlled data acquisition has made routine application of the method possible for a wide variety of gaseous ions. For example earlier work had shown that benzyl and tropylium ions can be characterized in a straightforward manner (Ref. 25); in recent work 12 isomers of the homologous $C_8H_9^+$ ions have been identified (Ref. 26), and 9 isomeric $C_3H_5O^+$ ions give characteristically different spectra (Ref. 27). The stability and formation of $C_2H_5S^+$ and $C_3H_7S^+$ ions show interesting differences as well as other behavior in parallel to that of the analogous O and N ions; the stability of the $CH_3CH_2S^+$ ion as indicated by appearance potentials, CA, and molecular orbital calculations is illustrative (Ref. 28 & 29).

One very promising research area for CA/MS is to supply structural information on large molecules in addition to that available from the EI mass spectrum. The CA spectra of fragment ions in the EI mass spectrum should be indicative of the structural relationships in the corresponding piece of the molecule. Such information should be especially valuable for substituents which have not had a large effect on the EI spectrum due to the presence of another substituent elsewhere in the molecule which directs the fragmentation more strongly. For example, CA spectra can indicate the amino acid sequences in fragment oligopeptide ions (Ref. 30). A recent study on CA experimental parameters (Ref. 31) points up a number of special considerations for the measurement of CA spectra of larger ions. An approximate method, based on Massey's adiabatic criterion (Ref. 32), has been devised to calculate the energy transfer frunction $P_C(E)$ for CA of ions. Results derived using this method are in surprisingly good agreement with experimental CA abundances for CH_4^+ ions from methane and and $C_7H_8^+$ ions from toluene at ion accelerating potential values (V_o) of 4.8 and 7.8 kV (Ref. 31). The calculated functions also rationalize well the unique insensitivity of the CA spectrum to ion internal energy. To maintain a constant $P_C(E)$ with increasing precursor ion mass it is necessary to increase V_o linearly; thus the same amount of internal energy will be transferred on collision to $\underline{m}/\underline{e}$ 100 ions at 8 kV as to $\underline{m}/\underline{e}$ 400 ions at 32 kV. Surprisingly, there is also a linear relationship between $P_C(E)$ and the ionization potential of the target; thus helium is by far the most efficient target gas, and metal surfaces gave negligible CA products in our experiments, contrary to previous findings (Ref. 33). We are now constructing a tandem double-focussing MS for high molecular weight CA studies to operate at V_o = 30 kV (Ref. 34).

Another potentially important application of CA and metastable ion (MI) mass spectra is "MS/MS" (Ref. 35 & 36); by analogy to GC/MS and LC/MS, this is defined as an analytical method in which mass spectrometry is used both for separation and for identification of the components of a mixture. Identification utilizes CA and MI mass spectra. The applicability and potentialities of MS/MS are complementary to GC/MS in terms of the sensitivity, selectivity, and value of the analytical information obtainable from a variety of organic mixtures. Different ionization methods are found to have complementary advantages. Interpretation of MI/CA mass spectra of molecular (M^+), $(M + H)^+$, and fragment ions using known correlations can provide valuable structural information, although computer matching of such spectra against reference electron-ionization mass spectra was relatively unsuccessful. As

examples of the specific detection of particular trace components in a complex mixture, thiophene, tetrahydropyran, and n-propylbenzene can be detected in gasoline at levels of <50 ppm, <50 ppm, and <0.1%, respectively (Ref. 36).

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