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Collisional activation mass spectra†

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Collision with gas molecules can be used to add internal energy to gaseous ions in transit through a mass spectrometer, causing their subsequent unimolecular decomposition. Mass analysis of the resulting fragment ions produces a collisional activation mass spectrum whose utility is basically similar to that of a normal mass spectrum. Promising applications to date have been found in ion structure characterization for fundamental studies and molecular structure determination, for which the insensitivity of the collisional activation mass spectrum to the ion's internal energy is a unique advantage; examples are given for structure determination of $C_7H_7^+$ and CSH_3^+ isomers. An additional application attracting increasing attention is as a separation/identification technique for complex mixtures; this involves mass spectrometric separation of the ionized mixture components followed by their identification from the corresponding collisional activation (or metastable ion) mass spectra. This two-dimensional mass spectrometry ('m.s.–m.s.') technique is complementary to g.c.–m.s. and liquid chromatography – mass spectrometry, and its use is illustrated by the determination of trace components in gasoline and the chirality of organophosphates.

Collisional activation is a method for adding sufficient internal energy to ions in transit through a mass spectrometer to cause their fragmentation; mass analysis of the resulting product ions thus produces a collisional activation mass spectrum of the particular ion species examined (McLafferty *et al.* 1973*a*, *b*; Levsen & Schwarz 1976; Kondrat & Cooks 1978; McLafferty 1977*a*). With instrumentation such as the reversed-geometry double-focusing mass spectrometer (Maurer *et al.* 1971; Cooks *et al.* 1973; Wachs *et al.* 1977) and linked-scan double focusing mass spectrometers (Weston *et al.* 1976; Beynon & Cooks 1976; Lacey & Macdonald 1977; Jennings 1977), a collisional activation mass spectrum can be obtained for most peaks (not less than about 1 %) in the normal mass spectrum. This extra dimension of mass spectral information has proved to be valuable for the characterization of gaseous ion structures, for molecular structure determination with the use of such fragment ion characterization, and for qualitative and quantitative analysis of complex mixtures. Similar applications are well known (Shannon & McLafferty 1966; Cooks *et al.* 1973; Wipf *et al.* 1973; Bowen & Williams 1977*a*) for the ionic products of metastable ion decompositions; particular advantages of collisional activation spectra are the much larger number of peaks, the formation of product ions from higher energy, loose complex reactions as well as low energy rearrangement reactions, and the fact that the relative abundances of collisional activation product ions produced by higher energy decompositions (those not exhibited by metastable ions) show a negligible dependence on their precursor ion's internal energy.

† Collisional activation and metastable ion characteristics, 68. For part 67, see Bockhoff & McLafferty (1979*b*).

The collisional activation process

When an ion of high translational energy (more than 1 keV) undergoes a grazing collision with a neutral species such as a helium atom, the interaction of the ion with the electron shell can change part of the ion's translational energy into internal energy. Collisions of multi-kilovolt energy ions result mainly in electronic excitation which can lead to decompositions requiring 10 eV or more of excitation energy; lower energy collisions cause mainly rotational and vibrational excitation. Recently Kim in our laboratory (1978) has used Massey's adiabatic criterion (Massey & Burhop 1952) to estimate the probability of adding particular values of energy to an ion on collision. The average energy added by collisional activation increases with increasing ion kinetic energy and decreases with ion mass; predictions based on such calculated functions for methane and toluene molecular ions gave semi-quantitative agreement with experiment. More importantly, these calculations justify the earlier experimental observations that the relative abundance of peaks in the collisional activation spectrum shows a negligible dependence on ion internal energy: exceptions are peaks representing product ions resulting from low energy decompositions, such as those observed for metastable precursor ions. The decrease in the average energy deposited by collisional activation with increasing ion mass emphasizes the importance of increasing the ion translational energy for studying larger molecules as well as for trace analysis.

Fragmentations of collisionally activated ions

The unimolecular decomposition of collisionally activated ions should follow the predictions of the quasi-equilibrium theory, and this appears to be generally true (McLafferty *et al.* 1973*a*; Levsen & Schwarz 1976; Cooks 1977*b*; Kim & McLafferty 1978*a*). However, the collisional activation mass spectrum of a molecular ion can be substantially different from that of the molecule's normal mass spectrum. Differences in product ion abundances arise mainly from different distributions of precursor ion internal energy values. The excited electronic states of the ion can have substantially different relative energies and are usually more dense than those of the corresponding molecule; it appears that the collisional activation energy deposition function is also more continuous (less discrete) than that from photoexcitation of ions, e.g. the butyrophenone molecular ion (Kim & McLafferty 1978*b*; Kim *et al.* 1978). This is due in part to the fact that the 3–8 keV ion accelerating potential usually used for collisional activation also causes a significant amount of vibrational excitation; multiple collisions will also tend to broaden the energy distribution of the resulting ions. The collisional activation mass spectrum of the phenol molecular ion is almost identical to the normal mass spectrum of phenol, as the electron removed from the π-system should not cause a large change in the electron energy levels. However, other substituted aromatic compounds show larger abundance differences for electron ionization *v.* collisional activation spectra, indicating substantial differences in electronic energy levels and/or excitation cross sections between the molecule and its ion (McLafferty *et al.* 1973*a*).

In some cases, there also appear to be differences in the ion decomposition pathways of the electron ionization spectrum and the collisional activation spectrum of the corresponding molecular ion. Because of the vertical ionization process, for fast (*ca.* 1 ps) electron ionization decompositions this could conceivably be due to the molecular geometry (bond distances and angles)

of the decomposing ion being more like the original molecule than the equilibrated ion structure which is subjected to collisional activation. This effect should be larger for functional groups such as the carbonyl group for which ionization should cause a large change in the equilibrium geometry, and substantial differences are found between the electron ionization and collisional activation spectra of alkanones. The longer time between ion formation and decomposition resulting from the collisional activation process also increases the chance for precursor ion isomerization, so that the resulting collisional activation spectrum represents that of the isomeric mixture. The precollision isomerization toluene$^{+\cdot}$ $\rightleftarrows$ cycloheptatriene$^{+\cdot}$ is well documented (McLafferty & Winkler 1974; Bockhoff & McLafferty 1979a, b), although this isomerization is also important in the electron ionization spectrum (Baldwin et $al.$ 1975). Although the loss of CH_2 from the molecular ion is almost never observed in a normal mass spectrum, it is commonly observed in collisional activation mass spectra of odd-electron ions such as the molecular ion of ethylene oxide (Van de Sande & McLafferty 1975), as well as even-electron ions (van de Graaf et $al.$ 1975). The loss of $CH_2^{\cdot\cdot}$ from the ethylene oxide molecular ion should be endothermic by 351 kJ/mol (Rosenstock et $al.$ 1977), and the photoelectron spectrum of ethylene oxide (at 14.2 eV) indicates that a substantial proportion of molecular ions of this energy are produced on ionization. Thus the absence of $CH_2O^{+\cdot}$ in the normal mass spectrum suggests that an additional reaction is possible on collisional activation of the molecular ion. As this would be a violation of the quasi-equilibrium theory, an alternative explanation is that in the $ca.$ 10^{-5} s before collision, isomerization of the molecular ion can produce open chain ions such as $CH_2{=}\overset{+}{O}CH_2^{\cdot}$ which should easily lose $CH_2^{\cdot\cdot}$ on excitation. Note that the loss of CH_2 does correlate qualitatively with ion structure, being far greater for ethylene oxide than for the isomeric acetaldehyde ($CH_3{-}CO{-}H^{+\cdot}$) and vinyl alcohol ($CH_2{=}CH{-}OH^{+\cdot}$) ions (Van de Sande & McLafferty 1975). Similarly, $CH_2^{\cdot\cdot}$ loss for the analogous even-electron ions exhibits an abundance order by collisional activation of protonated ethylene oxide > $CH_3O{=}CH_2^{+}$ > $CH_3CH{=}OH^{+}$ (Van de Graaf et $al.$ 1975). Correlation of the fragmentation behaviour of even-electron ions produced by collisional activation is proving of particular value to increase our understanding of normal mass spectral fragmentations, in which these ions are only produced as secondary ion products, which are thus difficult to study.

A very recent study by Franchetti et $al.$ (1978) gives evidence that it is also possible that ion fragmentations occur that are unique to the collision process itself. For protonated aromatic ions formed in a chemical ionization source, the fragmentations observed in photodissociation and collisional activation spectra are the same except for a dominant loss of H$\cdot$ in the collisional activation spectra. It was reasoned that this is not due to a low probability of photo-excitation production of ions with the average internal energy required for H$\cdot$ loss, but rather to either rotational energy deposited in the ion by collision, or collisional production of a triplet diradical. Presumably such a specifically excited ion would lose H$\cdot$ before energy randomization over the accessible energy levels of the ground electronic state. Such energy randomization is a basic assumption of the quasi-equilibrium theory, but other exceptions have been postulated (Andlauer & Ottinger 1971; Gross et $al.$ 1977; McAdoo et $al.$ 1978). However, in a comparison of the collisional activation and photodissociation spectra of protonated butyrophenone (Gooden & Brauman 1977; Kim & McLafferty 1978b; Kim et $al.$ 1978), substantial differences found were explained instead on the basis that photo-forbidden transitions were possible by collisional activation.

Collisional activation mass spectra v. *normal electron ionization mass spectra*

Thus generally the utility and applicability of collisional activation mass spectra are similar to normal electron ionization mass spectra. An unknown ion can be identified by matching its collisional activation spectrum with that of a reference ion spectrum: such reference data also make possible quantitative analysis for the ion. If a reference spectrum is not available for an unknown, interpretation of the spectrum's fragmentation behaviour can provide structural information. Labelling with stable isotopes is particularly helpful in such spectral interpretation. Thus any general familiarity the reader has with the applications of normal electron ionization spectra should be helpful in understanding the utility and potential of collisional activation mass spectrometry.

Kinetic energy release in collisional activation decompositions

Peak breadths in collisional activation or metastable ion spectra (Rickard *et al.* 1978) determined by ion energy analysis are dependent on the reverse activation energy as well as on the precursor ion internal energy in excess of the forward activation energy. The former should depend on the reaction, not the method of precursor ion excitation. However, the latter is minimized in metastable ion spectra (threshold energy decompositions), but could be substantial in collisional activation spectra, where the excess energy of the decomposing ion is mainly limited by the availability of competing dissociation channels with more favourable transition states (looser activated complexes). This not only produces generally broader peaks in collisional activation spectra than in metastable ion spectra, but also makes the peak widths of the former more dependent on the distribution of internal energy values of the precursor ion, making it more difficult to ascertain the portion of energy release due to reverse activation energy. A less important effect producing these broader peaks is that part of the ion's translational energy is converted into internal energy in the collisional activation process. Product ions resulting from a range of excitation energies will thus suffer translational energy losses over a range of values. Increasing the kinetic energy of the precursor ion reduces the relative effect of such peak broadening in energy analysis instruments, improving the resolution (Wachs *et al.* 1977). However, ion-momentum mass analysis of collisional activation spectra, such as with double-focusing (McLafferty 1977*b*) or quadrupole (Yost & Enke 1978) mass spectrometers appears to be a far better solution to the energy problem, and the narrower peaks should also bring a concomitant improvement in sensitivity.

Ion structure characterization

As discussed above, key advantages of the collisional activation spectrum for ion structure determination are its high information content and insensitivity to the internal energy of the ion, when peaks that are also found in the ion's metastable ion spectrum are neglected. This is not always true of other methods such as metastable ion spectra (Shannon & McLafferty 1966; Cooks *et al.* 1973) and ion–molecule reaction studies utilizing the ion cyclotron resonance (i.c.r.) spectrometer (Lehman *et al.* 1971). To minimize this problem in i.c.r. studies, the ions are made to undergo thermalizing collisions before reaction, but it is not always possible to establish the extent to which this has been successful before the reacting collisions take place. Ion photodissociation spectroscopy (Dunbar 1975) is a powerful new tool in which the absorption spectrum of an ion is determined by i.c.r. analysis of the resulting photodissociation products.

The applicability of collisional activation mass spectra to ion structure problems is shown by the wide variety of studies that have already been reported (McLafferty *et al.* 1973*b*; Levsen & Schwarz 1976; Cooks 1977*a*; Jennings 1977; McLafferty 1977*a*). In addition to the positive ions with a single charge, collisional activation spectra can be obtained from doubly charged ions and negative ions, or singly charged ions can be activated sufficiently to lose an electron to become doubly charged (Cooks *et al.* 1973). However, most collisional-activation structure studies have dealt with singly charged positive ions, mainly to distinguish between isomeric ions. For example, in a study of $C_8H_9^+$ ions, 13 isomers were identified (Köppel *et al.* 1977). A recent investigation by F. M. Bockhoff in our laboratory on the homologous $C_7H_7^+$ isomers tropylium, benzyl, and tolyl ions (Bockhoff & McLafferty 1979*a, b*) will be used to illustrate the applicability of this technique to such structure problems. The $C_7H_7^+$ isomers (see scheme 1) have been a problem of recurring interest for more than two decades since Meyerson and his colleagues (Rylander *et al.* 1957) postulated from labelling studies that toluene (**1**, Y = H), as well as cycloheptatriene (**2**, Y = H), molecular ions yielded tropylium ions (**4**, Y = H) instead of the expected benzyl ions (**3**, Y = H). In a variety of recent studies on this problem, which are reviewed elsewhere (McLafferty & Winkler 1974; Dunbar 1975; Dewar & Landman 1977; Bockhoff & McLafferty 1979*a, b*), the mechanistic picture of scheme 1 has been developed; probable heat of formation values in kilojoules per mole for the ions and transition states of the species Y = H (except **5** → **6**) are given in parentheses.
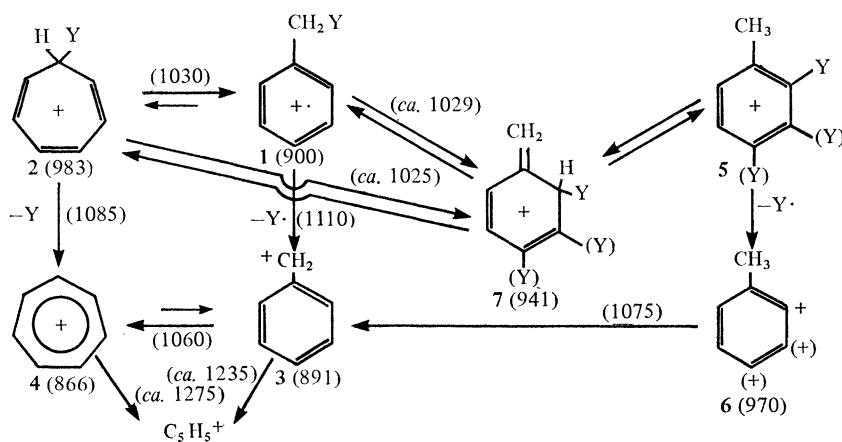
SCHEME 1

For the substituted toluene (**5**) and tolyl (**6**) ions other ring isomers are possible, and for the methylene-2,4-cyclohexadiene ion (**7**) the 2,5-isomer and Y-ring isomers are possible. Collisional activation mass spectra show that **1** and **2** ions give very similar proportions of **3** and **4** ions as a function of internal energy. Only the more stable **4** is formed at threshold decomposition energies through the prior equilibration **1** ⇌ **2**. In this, **1** is favoured; with increasing electron energy [3] increases to a maximum of 52 %, falling to 33 % for C_7H_8 ionization with 70 eV electrons because higher energy $C_7H_7^+$ ions undergo the equilibration **3** ⇌ **4**, favouring **4**. The ready equilibrations **1** ⇌ **2** and **3** ⇌ **4** compromise the preparation of pure **3** and **4** at both low and high energies; **4** ions can be purified by Dunbar's (1975) method of reacting the contaminant **3** ions with toluene. Essentially pure **3** are formed from benzyl fluoride (Y = F) at low ionizing energies because this **2** loses H, not F.

In a similar way, high purity *o*-, *m*-, and *p*-tolyl ions (**6**) are prepared by chemical ionization

of the corresponding fluorides (**5**, Y = F) as proposed by Leung *et al.* (1978). In electron ionization of $CH_3C_6H_4Y$, formation of the more stable **1** and **2** isomers usually accompanies **3–5**. Isomerization of low energy **5** to **7** appears to account for most **2** formation, while **1** mainly arises from the isomerization of **6** ions formed with higher internal energies through the intermediacy of **2** ions, consistent with the MINDO/3 calculations of Dewar & Landman (1977).

The chemistry of gaseous organic ions

Organic reactions proceeding through ionic mechanisms are of vital importance, and there is an extensive literature of solution studies in this area. However, the study of organic ions in the gas phase has the obvious advantage that there are no solvent effects; these can then often be deduced by difference in comparison to analogous solutions studies. As an example of this, Olah & Porter (1971) showed that in magic acid solution the ionization of α-phenethyl chloride produces the ethylenebenzenium and α-phenylethyl cations. Our collisional activation studies of gaseous ions (Köppel *et al.* 1977) showed that ionization of this precursor with low energy electrons only produces protonated benzocyclobutene, although the corresponding β-phenethyl bromide and iodide at low energies do indeed produce the ethylenebenzenium ion. A possible anchimeric-assisted reaction mechanism is proposed to account for these differences.

Ionic intermediates appear to be of particular importance for catalytic and high temperature reactions of hydrocarbons. Collisional activation studies on the unimolecular decompositions of a variety of hydrocarbon ions have now been published, and particular cases allow pertinent comparison to the corresponding condensed phase or high pressure gas phase reactions (McLafferty *et al.* 1973*b*; McLafferty & Winkler 1974; Levsen 1975; Levsen & Schwarz 1976; Dymerski & McLafferty 1976; Levsen & Heimbrecht 1977; Köppel *et al.* 1977).

Molecular structure determination

The substantial utility of high-resolution mass spectrometry (Beynon 1960) is due to the fact that it provides the *elemental composition* as well as the mass of the fragments of the unknown molecule shown in a mass spectrum. The use of collisional activation mass spectra for characterization of the *structure* of such fragments in an unknown mass spectrum can provide even more useful information, and should be especially important in the examination of complex molecules. For example, the m/z 45 (C_2H_5O) peaks in the mass spectra of 2-heptadecanol and 5α-pregnan-20β-ol-3-one gave collisional activation spectra consistent with the presence of $-CH(CH_3)OH$ groups in these molecules, while the m/z 45 in 1,2,3,5-tetra-*O*-methyl-β-ᴅ-ribofuranose gave a collisional activation spectrum consistent with $-CH_2OCH_3$ (McLafferty *et al.* 1973*b*). The collisional activation spectra of the $M^{+\cdot}$ (molecular ion) and $[M-H_2O]^{+\cdot}$ peaks in the cholesterol mass spectrum shows nearly identical abundances for methyl loss. This is consistent with the fact that the OH group is sufficiently removed from the sites of potential CH_3 loss that the structural change accompanying H_2O loss has little effect on CH_3 loss.

This utility of the collisional activation spectrum of a fragment ion in a normal mass spectrum to indicate the structural relation in the corresponding piece of the molecule can be especially valuable for substituents that have not had a large effect on the mass spectrum owing to the presence of another substituent elsewhere in the molecule which directs the primary fragmentation more strongly. For example, both metastable ion and collisional activation spectra can indicate the amino acid sequences in fragment oligopeptide ions (Wipf *et al.* 1973;

Levsen *et al.* 1974). In particular cases these data can differentiate isomeric leucine and iso-leucine residues which yield nearly identical normal mass spectra.

Bozorgzadeh *et al.* (1978) have recently described the use of such techniques for a detailed analysis of the mass spectra of 4-methylumbelliferone and 4,4-(1,2-dithiaphenyl)-5-hydroxy-nonane, with the collisional activation and metastable ion spectra of key fragment peaks providing definitive structural information. It is also pointed out that elemental composition information can be derived from the collisional activation spectrum of the ^{13}C isotope peak at $[M+1]^{+\cdot}$ if the $[M-H]^+$ peak is insignificant, as the ratio of peak heights of a particular fragment ion without and with the ^{13}C atom is directly related to the total number of carbon atoms in that fragment and in the molecule.

Fragmentation information of metastable ion and collisional activation spectra can also be of high utility if a 'soft' ionization method such as chemical or field ionization has been used. Levsen & Schulten (1976) used field ionization to obtain a relatively simple mass spectrum of a complex mixture produced by the pyrolysis of DNA; after separation of molecular ions of particular components their collisional activation spectra were obtained, from which component structures could be identified. A particularly impressive example has been reported recently by Burlingame (Straub *et al.* 1978) in which it was necessary to use the field desorption technique to obtain mass spectral data on polar high molecular mass covalent adducts formed by reacting 7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene with DNA *in vitro*. The principal peaks observed were the cationized molecules, $[M+Na]^+$, of the components, with no fragment information. A major peak at *m/z* 569 was shown to arise from the deoxyguanosine adduct. Its metastable ion spectrum showed only a single peak, while there were more than 15 peaks in the collisional activation spectrum. This fragmentation information could be related to the adduct structure, and was very similar to the normal mass spectrum of the permethylated deoxyguanosine adduct.

Complex mixture analysis by two-dimensional mass spectrometry

One of the most promising applications of collisional activation and metastable ion spectra is in a tandem mass analysis technique termed 'm.s.–m.s.' (McLafferty & Bockhoff 1978; Kondrat & Cooks 1978). By analogy with g.c.–m.s. and liquid chromatography–mass spectrometry, this is an analytical method in which mass spectrometry is used both for separation and for identification of components of a mixture. Conventional ionization methods are used to produce a mixture of ions representing the sample components; these ions are then separated mass spectrometrically according to their mass:charge ratio (*m/z*). The individual separated ions are then identified or determined quantitatively with the use of their collisional activation or metastable ion spectra. The applicability and potentiality of this tandem mass spectrometric technique are complementary to g.c.–m.s. in terms of the sensitivity, selectivity, and specificity of the analytical information obtainable from a variety of organic mixtures. Extensive reviews of the methodology and applications of this method have recently appeared (Kondrat & Cooks 1978; McLafferty & Bockhoff 1978), to which the reader is referred for more detailed information. Analyses of a surprising variety of complex mixtures have been reported recently (Wipf *et al.* 1973; Smith *et al.* 1974; Levsen & Schulten 1976; Cooks 1977*a*; Brent *et al.* 1977; McReynolds & Anbar 1977; Straub *et al.* 1978; Maquestiau *et al.* 1978; Kondrat *et al.* 1978*a, b*; Abbott *et al.* 1978).

In comparing g.c.–m.s. and liquid chromatography – mass spectrometry with this tandem technique, a mass spectrometer can accept samples of much lower volatility than can g.c., although not liquid chromatography. Further, the time required for mass separation (*ca.* 10^{-5} s) is not only far shorter than the many minutes required for chromatographic separation but is sufficiently brief for most applications where continuous analysis is necessary. A distinct disadvantage of the tandem mass spectrometry technique is that most ionization methods do not produce a single peak to correspond to each mixture component, as do chromatographic separation methods; an electron ionization mass spectrum of a pure compound can have hundreds of peaks, while 'gentle' ionization methods such as chemical, field, or low voltage electron ionization could produce no peaks for particular components. Another handicap at present is that collisional activation and metastable ion reference mass spectral files are extremely limited in comparison with the large data bases available for normal mass spectra, and that relatively poor results are achieved in matching collisional activation mass spectra against the electron ionization reference file (McLafferty & Bockhoff 1978). Of particular interest is the collision method developed by Bowie & Blumenthal (1975) in which two electrons are removed from a negative ion to form a positive ion; a substantial proportion of the resulting ions have sufficient internal energy to undergo further decomposition. Thus these '$+E$' spectra of negative molecular ions resemble normal electron ionization spectra in that the relative abundance of the positively charged precursor ion (e.g. a molecular ion) is dependent on its relative stability.

Automated computer systems for data acquisition and reduction, which have become almost a necessity for g.c.–m.s. systems, are still not common for the tandem mass spectrometry technique, although those in use generally show the same advantages and potentialities that are well known for g.c.–m.s. systems (Wachs *et al.* 1977). 'Multiple ion monitoring', in which the second mass spectrometer is focused sequentially on the few peaks most characteristic of the sought component ion separated by the first mass spectrometer, should prove of critical value for quantitative analysis of predetermined mixture components (Wipf *et al.* 1973; McLafferty & Bockhoff 1978), as is already well established for g.c.–m.s. and liquid chromatography–mass spectrometry. The use of high resolution (McLafferty 1977*b*) should greatly improve mass spectrometry as a separation method. In general, mass separation is complementary to chromatographic methods, and thus should find additional applications. Of course a combination of these methods ('g.c.–m.s.–m.s.' or 'l.c.–m.s.–m.s.') should provide even more separation specificity.

At present, probably the most promising applications of tandem mass spectrometry separation/identification systems are to the detection and quantitative determination of particular trace components in complex mixtures, as the ionization method can be optimized for that component and its collisional activation or metastable ion spectrum can be determined from a standard under the same conditions. For example, 'regular' petrol, which gives a peak in its normal mass spectrum at almost every mass value up to m/z 140, can be analysed for trace levels of thiophene, tetrahydropyran and *n*-propylbenzene at levels of less than 50 parts/10^6, less than 50 parts/10^6, and less than 0.1 %, respectively (McLafferty & Bockhoff 1978). The metastable ion spectrum is more characteristic for the detection of the last substance, while high sensitivities were achieved for the second component despite the substantial interference from hexanes at m/z 86 by using the gasoline sample as the ionizing reagent gas for chemical ionization.

A particularly intriguing application of this technique is a new method for determining the chirality at phosphorus by using ^{16}O, ^{17}O, ^{18}O-labelled phosphate monoesters developed by Knowles and associates (Abbott *et al.* 1978). Chromatographic separation of diastereomeric cyclic methyl esters gives the *syn* and *anti* form, each containing three species with the label pairs ^{16}O–^{17}O, ^{17}O–^{18}O and ^{18}O–^{16}O. Tandem mass spectrometry is used to separate fragment ions corresponding to each of the species, for which subsequent metastable ion fragmentation losing CH_2O identifies the oxygen isotope in the methoxyl group. These data provide unequivocal evidence of the original chirality at phosphorus.

References (McLafferty)

Abbott, S. J., Jones, S. R., Weinman, S. A. & Knowles, J. R. 1978 *J. Am. chem. Soc.* **100**, 2558–2560.

Andlauer, B. & Ottinger, C. 1971 *J. chem. Phys.* **55**, 1471.

Baldwin, M. A., McLafferty, F. W. & Jerina, D. M. 1975 *J. Am. chem. Soc.* **97**, 6169–6174.

Beynon, J. H. 1960 *Mass spectrometry and its applications to organic chemistry.* Amsterdam: Elsevier.

Beynon, J. H. & Cooks, R. G. 1976 *Int. J. Mass Spectrom. Ion Phys.* **19**, 107.

Bockhoff, F. M. & McLafferty, F. W. 1979*a* *J. Am. chem. Soc.* **101**, 1783–1786.

Bockhoff, F. M. & McLafferty, F. W. 1979*b* *Org. Mass Spectrom.* **14**, 181–184.

Bowen, R. D. & Williams, D. H. 1977*a* In *Chemical applications of high-performance mass spectrometry* (ed. M. L. Gross), pp. 18–46. Washington: American Chemical Society.

Bowie, J. H. & Blumenthal, T. 1975 *J. Am. chem. Soc.* **97**, 2959–2960.

Bozorgzadeh, M. H., Morgan, R. P. & Beynon, J. H. 1978 *Analyst* **103**, 613–622.

Brent, D. A., Bugge, C. J., Cuatrecasas, P., Holbert, B. S., Nelson, D. J. & Sahyoun, N. 1977 In *Chemical applications of high-performance mass spectrometry* (ed. M. L. Gross), pp. 229–247. Washington: American Chemical Society.

Cooks, R. G. 1977*a* In *Chemical applications of high-performance mass spectrometry* (ed. M. L. Gross), pp. 58–79. Washington: American Chemical Society.

Cooks, R. G. 1977*b* *Collision spectroscopy.* New York: Plenum Press.

Cooks, R. G., Beynon, J. H., Caprioli, R. M. & Lester, G. R. 1973 *Metastable ions.* Amsterdam: Elsevier.

Dewar, M. J. S. & Landman, D. 1977 *J. Am. chem. Soc.* **99**, 2446–2453.

Dunbar, R. C. 1975 *J. Am. chem. Soc.* **97**, 1382–1384.

Dymerski, P. P. & McLafferty, F. W. 1976 *J. Am. chem. Soc.* **98**, 6070–6072.

Franchetti, V., Freiser, B. S. & Cooks, R. G. 1978 *Org. Mass Spectrom.* **13**, 106–110.

Gooden, R. & Brauman, J. I. 1977 *J. Am. chem. Soc.* **99**, 1977.

Gross, M. L., Russell, D. H., Aerni, R. J. & Bronczyk, S. A. 1977 *J. Am. chem. Soc.* **99**, 3603.

Jennings, K. R. 1977 In *Chemical applications of high-performance mass spectrometry* (ed. M. L. Gross), pp. 3–17. Washington: American Chemical Society.

Kim, M. S., Dunbar, R. C. & McLafferty, F. W. 1978 *J. Am. chem. Soc.* **100**, 4600–4601.

Kim, M. S. & McLafferty, F. W. 1978*a* *J. Am. chem. Soc.* **100**, 3279–3282.

Kim, M. S. & McLafferty, F. W. 1978*b* *J. phys. Chem.* **82**, 501–504.

Kondrat, R. W. & Cooks, R. G. 1978 *Analyt. Chem.* **50**, 81A.

Kondrat, R. W., Cooks, R. G. & McLaughlin, J. C. 1978*a* *Science, N.Y.* **199**, 978–980.

Kondrat, R. W., McClusky, G. A. & Cooks, R. G. 1978*b* *Analyt. Chem.* **50**, 1222–1223.

Köppel, C., Van de Sande, C. C., Nibbering, N. M. M., Nishishita, T. & McLafferty, F. W. 1977 *J. Am. chem. Soc.* **99**, 2883–2888.

Lacey, M. J. & Macdonald, C. G. 1977 *Org. Mass Spectrom.* **12**, 587.

Lehman, T. A., Elwood, T. A., Bursey, J. T., Bursey, M. M. & Beauchamp, J. L. 1971 *J. Am. chem. Soc.* **93**, 2108.

Leung, H.-W., Ichikawa, H., Li, Y.-H. & Harrison, A. G. 1978 *J. Am. chem. Soc.* **100**, 2479–2484.

Levsen, K. 1975 *Tetrahedron* **31**, 2431–2433.

Levsen, K. & Heimbrecht, J. 1977 *Org. Mass Spectrom.* **12**, 131–135.

Levsen, K. & Schulten, H.-R. 1976 *Biomed. Mass Spectrom.* **3**, 137.

Levsen, K. & Schwarz, H. 1976 *Angew. Chem. int. Edn* **15**, 509.

Levsen, K., Wipf, H.-K. & McLafferty, F. W. 1974 *Org. Mass Spectrom.* **8**, 117–128.

Maquestiau, A., Van Haverbeke, Y., Flammang, R., Mispreuve, H., Kaisin, M., Braekman, J. C., Daloze, D. & Tursch, B. 1978 *Steroids* **31**, 31–48.

Massey, H. S. W. & Burhop, E. H. S. 1952 In *Electronic and ionic impact phenomena*, p. 513. London: Oxford University Press.

Maurer, K. H., Brunee, C., Kappus, G., Habfast, K., Schröder, U. & Schulze, P. 1971 *19th Annual Conference on Mass Spectrometry and Allied Topics*, Atlanta, Paper K-9.

McAdoo, D. J., Witiak, D. M., McLafferty, F. W. & Dill, J. D. 1978 *J. Am. chem. Soc.* **100**, 6639–6643.

McLafferty, F. W. 1977a In *Chemical applications of high-performance mass spectrometry* (ed. M. L. Gross), pp. 47–57. Washington: American Chemical Society.

McLafferty, F. W. 1977b In *Analytical pyrolysis* (ed. C. E. R. Jones & C. A. Cramers), pp. 39–48. Amsterdam: Elsevier.

McLafferty, F. W. & Bockhoff, F. M. 1978 *Analyt. Chem.* **50**, 69–76.

McLafferty, F. W. & Winkler, J. 1974 *J. Am. chem. Soc.* **96**, 5182–5189.

McLafferty, F. W., Bente, P. F., Kornfeld, R., Tsai, S.-C. & Howe, I. 1973a *J. Am. chem. Soc.* **95**, 2120–2129.

McLafferty, F. W., Kornfeld, R., Haddon, W. F., Levsen, K., Sakai, I., Bente, P. F., Tsai, S.-C. & Schuddemage, H. D. R. 1973b *J. Am. chem. Soc.* **95**, 3886–3892.

McReynolds, J. H. & Anbar, M. 1977 *Int. J. Mass Spectrom. Ion Phys.* **24**, 37–49.

Olah, G. A. & Porter, R. D. 1971 *J. Am. chem. Soc.* **93**, 6877.

Rickard, G. J., Cole, N. W., Christie, J. R. & Derrick, P. J. 1978 *J. Am. chem. Soc.* **100**, 2904–2905.

Rosenstock, H. M., Draxl, K., Steiner, B. W. & Herron, J. T. 1977 *J. phys. Chem. Ref. Data* **6**, Suppl. 1.

Rylander, P. N., Meyerson, S. & Grubb, H. M. 1957 *J. Am. chem. Soc.* **79**, 842.

Shannon, T. W. & McLafferty, F. W. 1966 *J. Am. chem. Soc.* **88**, 5021–5022.

Smith, D. H., Djerassi, C., Maurer, D. H. & Rapp, O. 1974 *J. Am. chem. Soc.* **96**, 3482.

Straub, K., Meehan, T., Kambara, H., Burlingame, A. L. & Evans, S. 1978 *Am. Soc. for Mass Spectrom. Conf.*, St Louis.

van de Graaf, B., Dymerski, P. P. & McLafferty, F. W. 1975 *J. chem. Soc. chem. Commun.*, pp. 978–979.

Van de Sande, C. C. & McLafferty, F. W. 1975 *J. Am. chem. Soc.* **97**, 4613–4616.

Wachs, T., Van de Sande, C. C., Bente, P. F., Dymerski, P. P. & McLafferty, F. W. 1977 *Int. J. Mass Spectrom. Ion Phys.* **23**, 21–27.

Weston, A. F., Jennings, K. R., Evans, S. & Elliot, R. M. 1976 *Int. J. Mass Spectrom. Ion Phys.* **20**, 317.

Wipf, H.-K., Irving, P., McCamish, M., Venkataraghavan, R. & McLafferty, F. W. 1973 *J. Am. chem. Soc.* **95**, 3369–3375.

Yost, R. A. & Enke, C. G. 1978 *J. Am. chem. Soc.* **100**, 2274–2275.