The Use of Two Linked Scanning Modes in Alternation to Analyse Metastable Peaks

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Analyses of masses and relative kinetic energies for metastable peaks may be achieved by the use in alternation of two types of linked scans of the electric sector voltage and magnetic field. The method is demonstrated by an appraisal of hydrogen exchange in molecular ions of 2-methyl-[²H₃]-benzoic acid.

INTRODUCTION

Identification of the daughter ions formed in the flight tube of a double focusing mass spectrometer from a selected parent ion (constant parent ion spectrum) can be used to establish molecular and ionic structure¹ and to resolve mixtures without prior separation.^{2,3} Analyses of masses and relative kinetic energies for the daughter ions are achieved simultaneously in the constant parent ion spectrum (obtained by an E scan) for fragmentations within the second field free region (2nd FFR) of an instrument of reversed geometry. Release of kinetic energy, however, often obscures both the mass analysis of these daughter ions and the evaluation of peak shapes and areas, though methods for reducing these difficulties have been mooted.4,5 The effective mass resolution for daughter ions is improved, because of kinetic energy discrimination, for fragmentations within the 1st FFR of an instrument of conventional or reversed geometry,^{6,7} and we have recently described procedures by which analyses of the masses and kinetic energy profiles for the metastable peaks can be achieved by performing a B,E scan (constant parent ion spectrum) and localized V scans (constant daughter ion spectra) in alternation.⁸ We now describe an improved procedure using a B,E scan and localized E,B^2 scans (constant daughter ion spectra) in alternation, with minimal reference to main beam ions, to achieve analyses of masses and relative kinetic energies for a series of metastable peaks or collision induced peaks. The method may be applied to the characterization of selected reactions related by the same parent ion, same daughter ion or same neutral fragment.

An appreciation of the contrasting profiles of metastable peaks provided by various modes of simple and linked scanning, and of the interrelationships between the scanning modes, can be gained from a three-dimensional diagram.⁹ Symbols and nomenclature used in the present paper have been defined previously.⁹

It has been shown that site-specific equilibration of the methyl and carboxyl hydrogen atoms of 2methylbenzoic acid precedes water loss from $[M]^{++}$ ions, and a hydrogen isotope effect is involved for metastable ion transitions.¹⁰ In the present study we illustrate the use of two linked scanning modes in alternation in an investigation of the hydrogen exchange at different levels of internal energy of the $[M]^{+\cdot}$ ions.

EXPERIMENTAL

The spectra were determined on a VG Micromass 70-70 mass spectrometer, an instrument of conventional geometry, at an accelerating voltage of 4 kV and an electron energy of 70 eV. Our instrument has a fixed energy resolving slit $(\delta\rho/\rho = 0.01)$, and a collision cell is located immediately following the object slit for the electric sector. Helium was used when required in the collision cell at a pressure of approximately 0.04 Pa. Samples were introduced by direct insertion.

The linked scans available on the instrument are the B,E scan and E,B^2 scan, in which an appropriate relationship is maintained between E and values of B measured by means of a Hall probe. The output of the Hall probe drives the mass marker in the mode of B,E scanning to provide a scale of μ (mass of daughter ion) on the galvanometer charts. A digital voltmeter displays measurements of V, E or μ during the B,E scan. In the mode of E,B^2 scanning, the scale markings on the galvanometer charts are proportional to E and hence to ρ (relative kinetic energy), while the voltmeter also provides a digital display of values for E during the scan.

2-Methyl-[²H₃]-benzoic acid

Methyl 2-aminobenzoate was reduced to 2-methyl-[${}^{2}H_{3}$]-aniline with a solution of lithium aluminium deuteride (Merck, 99.5 atom % ${}^{2}H$) and aluminium chloride in dibutyl ether, using a method described for the unlabelled compound.¹¹ The diazonium salt of 2methyl-[${}^{2}H_{3}$]-aniline was converted to the nitrile and hydrolysed to yield 2-methyl-[${}^{2}H_{3}$]-benzoic acid (98% ${}^{2}H_{3}$, 2% ${}^{2}H_{2}$; low eV mass spectrum).

The resolution of masses and relative kinetic energies of daughter ions of a selected parent ion may be illustrated by the following procedure ((a) to (e)) for analysis of peaks for water loss in the 1st FFR from $[M]^{+\cdot}$ ions of 2-methyl- $[{}^{2}H_{3}]$ -benzoic acid.

(a) Establishing reference peak in B, E scan. The $[M]^+$ ion of m/z 139 u was focused using the oscilloscope screen, and V and E were placed under manual control in preparation for a B,E scan. The spectrum for $\mu = 138-140$ u was displayed on the oscilloscope using repeated B, E scans and V was finely adjusted to minimize any contributions from adjacent main beam peaks, for example at $\mu = 138$ u and 140 u. The metastable peak for $m/z \ 139 \ u \rightarrow m/z \ 119 \ u$ was selected as the reference and its peak centre at $\mu = 119$ u in the B,E scan was displayed on the oscilloscope, using the mass indicator reading as a guide. The reference peak was used to calibrate the mass marker and the parent ions for the peak could be confirmed by a localized V scan, as indicated previously.⁸ The multiplier gain and the rate and range of the B,E scan were adjusted to monitor the group of peaks of interest ($\mu = 119$ -122 u) on the galvanometer chart. The oscilloscope again displayed the centre of the reference peak at the completion of this localized B, E scan.

(b) Switching from B,E scan to E,B^2 scan. The value of E for the centre of the reference peak was noted (E_1) and the mode was switched to an E,B^2 scan. E was adjusted to E_1 to return the centre of the peak to the screen.

(c) Calibration of the kinetic energy scale. A localized E, B^2 scan over the range preset under (a) above displayed the kinetic energy profile of the reference metastable peak. The oscilloscope again displayed the centre of the reference peak at the completion of this scan. The energy scale provided by the mass marker on the galvanometer chart was given numerical values by using the reading of the 'mass' indicator for the centre of the reference peak as a guide.

(d) Localized E, B^2 scans for adjacent peaks. E was reduced to 119/120 of E_1 at constant B and V, and the scan was initiated to display the energy profile of the metastable peak for daughter ions of m/z 120 u. Although this peak was displaced along the chart relative to the position of the reference peak, the energy scale remained constant because V was constant. Similarly, reduction of E to 119/121 of E_1 and 119/122 of E_1 followed by the localized E, B^2 scans yielded the energy profiles of any metastable peaks for daughter ions of m/z 121 u and 122 u, respectively. E was then increased to E_1 to return the reference peak centre to the oscilloscope screen and to ensure that its height did not change significantly during the course of these operations. If the peak centres appear on the calibrated scale of the galvanometer charts at R_{119} , R_{120} , R_{121} and R_{122} , the m/z values of the parent ions for the adjacent peaks are

$$\begin{array}{l} (120 \times R_{119} \times 139) / (R_{120} \times 119) \, u, \\ (121 \times R_{119} \times 139) / (R_{121} \times 119) \, u \\ \text{and} \, (122 \times R_{119} \times 139) / (R_{122} \times 119) \, u. \end{array}$$

The sequence of measurements was repeated in the presence and absence of helium in the collision cell so that corrections could be applied for contributions to the collision induced peaks from any underlying metastable peaks. In addition, the helium pressure could be finely adjusted to maximize the contributions to the peak of the collision induced processes.

(e) Switching from E,B^2 scan to B,E scan. The reference metastable peak was returned to the oscilloscope screen at $E = E_1$ and the mode was switched to B,E scanning. E was readjusted to E_1 to display the centre of the reference peak on the screen in the B,E scan for $[M]^{+\cdot}$ ions.

The resolution of masses and relative kinetic energies for daughter ions of a series of adjacent parent ions may be illustrated by the following procedure for analysis of the metastable peaks for CO loss from $[M-hydroxyl]^+$ and $[M-water]^+$ ions. A B,E scan for parent ions of m/z 119 u was established, using an analogous procedure to that described in (a) above, and the metastable peak for m/z 119 u $\rightarrow m/z$ 91 u was selected as the reference peak. The mode was switched to E, B^2 scanning and kinetic energy profiles of the metastable peaks for daughter ions of m/z91–94 u were obtained by localized E, B^2 scans, using analogous procedures to those indicated above for daughter ions of m/z 119–122 u. The kinetic energy profiles of the peaks for CO loss from metastable [M-hydroxyl]⁺ ions were significantly broader than those for CO loss from metastable $[M-water]^{+}$ ions.

RESULTS AND DISCUSSION

Daughter ion distributions for selected reactions of 2-methylbenzoic acid and 2-methyl- $[{}^{2}H_{3}]$ -benzoic acid in the source at 70 eV are shown in Table 1. Table 2 shows the relative areas of metastable peaks and collision induced peaks for the same fragmentations in the 1st FFR, evaluated by the procedures outlined in the Experimental section.

Loss of hydroxyl from $[M]^{+}$ ions of an aromatic carboxylic acid does not normally involve a primary hydrogen isotope effect¹² and therefore the extent of exchange for the various internal energy windows can be assessed from the relative abundances of [M-OH]⁺ and $[M-OD]^+$ ions for 2-methyl- $[^2H_3]$ -benzoic

Table 1. Partial mass spectra^a of 2-methylbenzoic acid and 2-methyl- $[{}^{2}H_{3}]$ -benzoic acid

m/z	2-Methyl benzoic acid	2-Methyl-[² H ₃]- benzoic acid	m/z	2-Methyl benzoic acid	2-Methyl-[² H ₃]- benzoic acid
139		94	118	81	
138		5	95		1
137		1	94		30
136	96		93		26
135	4		92	1	23
122		9	91	52	17
121		13	90	37	3
120		45	89	10	
119	19	33			

^a Peak heights for the 70 eV mass spectra are corrected for the presence of natural isotopes but not for 2% ²H₂ impurity. The heights are the mean of three scans and are expressed as percentages of the total for that group of peaks.

Com- pound	Parent ion	Daughter ion	Peak area ^a
2-Methyl			
benzoic acid	Metastable (¹³ C)[M] ⁺	[(¹³ C)M-H ₂ O]+·	8 ⁶
	Metastable [M]+-	[M-H ₂ 0] ^{+. –}	92
	Collisionally activated [M] ⁺⁻	[M-OH]+	9 °
	Collisionally activated [M] ⁺⁻	[M–H ₂ O] ⁺⁻	91ª
	Metastable [M–OH]+	[M-OH-CO]+	35°
	Metastable [M−H₂O]+·	[M-H ₂ O-CO] ^{+.}	65
2-Methyl-[² H ₂]-			
benzoic acid	Metastable [M]+·	[M-H-O]+·	0°
	Metastable [M]+·	[M-HOD]+·	61°
	Metastable [M]+-	[M-D ₂ O]+·	39
	Collisionally activated [M] ⁺	[M-OH]+	7°
	Collisionally activated [M] ⁺⁺	[M-OD] ⁺	16°
	Collisionally activated [M]+-	[M-HOD]+·	41 ^{c,d}
	Collisionally activated [M]+-	[M-D ₂ O]+-	36 ^d
	Metastable	[M-OH-CO]+	17°
	Metastable [M-OD]+	[M-OD-CO]+	22 ^c
	Metastable [M−HOD]**	[M-HOD-CO]+·	34 ^c
	Metastable [M-D ₂ O] ⁺⁻	[M-D ₂ O-CO] ⁺⁻	27

Table 2. Reactions in the 1st FFR of 2-methylbenzoic acid and 2-methyl- $[{}^{2}H_{3}]$ -benzoic acid

^a The peak areas are the mean of four localized *E*,*B*² scans and are expressed as percentages of the total for that group of peaks.

^b Area calculated from 12 C data = 8; thus contribution of $[M-OH]^+$ ions from metastable $[M]^{++}$ ions is negligible.

^e After subtraction of contribution from reaction of ions containing natural isotopes.

^d After subtraction of contribution from metastable peak.

acid. The extent of exchange prior to hydroxyl loss increases from 62% for $[M]^{+\cdot}$ ions with sufficient average internal energy to lose both hydroxyl and CO in the source to 79% for $[M]^{+\cdot}$ ions with sufficient energy to lose only hydroxyl in the source. $[M]^{+\cdot}$ ions of intermediate energy that lose hydroxyl in the source and CO in the 1st FFR show an intermediate extent of exchange (75%), while $[M]^{+\cdot}$ ions of lower energy that lose hydroxyl in the 1st FFR after collisional activation show almost complete exchange (93%). The results demonstrate that the extent of exchange increases with decrease in average internal energy (longer lifetime) of the $[M]^{+\cdot}$ ions.

Although equal losses of HOD and D_2O from the $[M]^{+\cdot}$ ions of 2-methyl- $[^2H_3]$ -benzoic acid would be expected after equilibration of the methyl and carboxyl hydrogen atoms, HOD loss will be favoured both by incomplete exchange and by the primary hydrogen isotope effect.¹⁰ Since the isotope effect will vary with average internal energy of the parent ions,¹³ the ratio of $[M-HOD]^{+\cdot}$ to $[M-D_2O]^{+\cdot}$ ions is expected to decrease with decreasing energy of the $[M]^{+\cdot}$ ions (increased extent of exchange) and then to in-

crease at threshold internal energies (increased isotope effect).

For unstable $[M]^{+\cdot}$ ions with sufficient internal energy to undergo consecutive losses of water and CO in the source, the estimate of the ratio is obscured by subsequent loss of H from the granddaughter ions (Table 1). By assuming as a first approximation that the relative ion currents for the reactions are the same for the labelled and unlabelled compounds, values of the ratio can be deduced for $[M]^{+\cdot}$ ions that undergo consecutive losses of water and CO in the source (1.8) and also for less stable $[M]^{+\cdot}$ ions that undergo subsequent loss of H in the source (2.0).

The ratio for more stable $[M]^{+\cdot}$ ions that lose water in the source and CO in the 1st FFR is estimated to be 1.26, while the ratio for similar $[M]^{+\cdot}$ ions that lose water in the source but do not lose CO is 1.36. The ratio for less energetic metastable $[M]^{+\cdot}$ ions that lose water in the 1st FFR is 1.56. The trend in these ratios conforms with expectations.

The ratio for collision induced loss of water from stable $[M]^{++}$ ions in the 1st FFR is 1.14. Primary isotope effects should be smaller for a collision induced fragmentation than for the metastable ion transition and this is reflected in the difference of the ratios (1.14 and 1.56, respectively). In addition, the ratio for the collision induced water loss from stable $[M]^{++}$ ions indicates a closer approach of the exchange to equilibrium than for $[M]^{++}$ ions undergoing the same reaction in the ion source, in agreement with the results found for hydroxyl loss. The equilibration may occur in the long-lived $[M]^{++}$ ions before collisional activation, but the results are also consistent with the lower average internal energies of the $[M]^{++}$ ions after collision compared with $[M]^{++}$ ions that undergo the same reactions in the source at 70 eV.

The exchange process in $[M]^{+}$ ions of 2-methylbenzoic acid is faster than the exchange of *ortho* and carboxyl hydrogen atoms in the $[M]^{+}$ ions of benzoic acid, since the exchange for the latter case is not apparent in stable [M]+ ions sampled by collisional activation.⁸ The hydrogen isotope effect operating in the water loss from [M]⁺⁻ ions of pyridine 4-carboxylic acid increases with decreasing internal energy of the parent ions,¹⁴ in agreement with the above data. It has been suggested that the ratedetermining step for water loss from [M]+ ions of 2-methylbenzoic acid is hydrogen transfer to the hydroxyl group,¹⁵ while the relatively large energy release for the water loss, compared with, say, 2hydroxybenzoic acid, is consistent with the proposal of a product-like transition state.¹⁶ Our observation that hydrogen exchange of the ortho substituents with the aromatic ring does not occur over the present timescale is consistent with analogous results for [M]⁺ ions of 2-hydroxybenzoic acid¹⁷ and phthalic acid.¹

Our analyses of metastable peaks and collision induced peaks were performed on an instrument with a wide energy resolving slit, and hence at good sensitivity, for the reactions in the 1st FFR, but the effective mass resolution of parent ions can be low under these conditions.^{6.7} The mass resolution was not a problem in the present examples because the peak profiles in the localized E,B^2 scans can be readily corrected for overlapping contributions from fragmentation of ¹³C parent ions. Furthermore, the molecules are of high isotopic purity and fragmentation of $[M-H]^+$ ions is not significant. Higher energy resolution may be required to resolve the overlapping peaks for daughter ions in those cases where fragmentation of adjacent parent ions is important or where the parent molecules are not of high isotopic purity.

The fact that the effective mass resolution is high for daughter ions of reactions in the 1st $FFR^{6,7,19,20}$ is an advantage when using *B*,*E* scans for the direct analysis

of mixtures in conjunction with improved methodology for generating stable ions in the source for each component of the mixture. Although these considerations apply equally to instruments of conventional and reversed geometry, an advantage for direct mixture analysis in instruments of conventional geometry over those of reversed geometry²¹ is that daughter ions from fragmentation in the 2nd FFR are transmitted with the main beam for the former instrument and therefore cannot obscure the peaks for fragmentation in the 1st FFR.

REFERENCES

- K. Levsen and H. Schwarz, Angew. Chem. Int. Ed. Engl. 15, 509 (1976).
- 2. R. W. Kondrat and R. G. Cooks, Anal. Chem. 50, 81A (1978).
- 3. F. W. McLafferty and F. M. Bockhoff, Anal. Chem. 50, 69 (1978).
- F. W. McLafferty, in *Analytical Pyrolysis*, ed. by C. E. R. Jones and C. A. Cramers, pp. 39–48. Elsevier, Amsterdam, (1977).
- 5. H. H. Tuithof, Int. J. Mass Spectrom. Ion Phys. 23, 147 (1977).
- M. J. Lacey and C. G. Macdonald, Org. Mass Spectrom. 13, 243 (1978).
- 7. W. F. Haddon, Anal. Chem. 51, 983 (1979).
- M. J. Lacey and C. G. Macdonald, Org. Mass Spectrom. 14, 465 (1979).
 M. J. Lacey and C. G. Macdonald, Org. Mass Spectrom. 12,
- 5. M. J. Lacey and C. G. Macdonald, Org. Mass Spectrom. 12, 587 (1977).
- M. J. Lacey, C. G. Macdonald and J. S. Shannon, Org. Mass Spectrom. 5, 1391 (1971).
- 11. R. F. Nystrom and C. R. A. Berger, J. Am. Chem. Soc. 80, 2896 (1958).
- I. Howe and F. W. McLafferty, J. Am. Chem. Soc. 92, 3797 (1970).

- I. Howe and F. W. McLafferty, J. Am. Chem. Soc. 93, 99 (1971).
- R. Neeter and N. M. M. Nibbering, Org. Mass Spectrom. 5, 735 (1971).
- 15. M. A. Winnik, Org. Mass Spectrom. 9, 920 (1974).
- M. L. Gross, F. L. DeRoos and M. K. Hoffman, Org. Mass Spectrom. 12, 258 (1977).
- 17. S. A. Benezra and M. M. Bursey, Org. Mass Spectrom. 6, 463 (1972).
- J. L. Holmes and F. Benoit, Org. Mass Spectrom. 4, 97 (1970).
- D. S. Millington and J. A. Smith, Org. Mass Spectrom. 12, 264 (1977).
- 20. R. S. Stradling, K. R. Jennings and S. Evans, Org. Mass Spectrom. 13, 429 (1978).
- T. Ast, M. H. Bozorgzadeh, J. L. Wiebers, J. H. Beynon and A. G. Brenton, Org. Mass Spectrom. 14, 313 (1979).

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