Dear Sir

A Rapid Method for the Generation of Multiple Constant Parent Ion Spectra

Linked scans of the magnetic field (B) and the electric sector voltage (E) at constant accelerating voltage (V) to produce spectra containing only ions originating in the 1st field free region (FFR) of double focusing mass spectrometers are well known.[1,2] Three-dimensional representation by various techniques using more than one scanning mode has been shown to provide the maximum amount of information about metastable peaks for cases where sample size is not limiting.[3-5] Computer techniques now also allow for automatic switching to linked scans during normal magnet scans, using previously entered calibration files for each desired daughter or precursor.[6]

We would like to point out a simplified procedure for the acquisition of all desired constant parent ion spectra for a given sample, based on the linked scan at constant B/E. Normally, individual scans would be carried out for each parent ion using different B/E ratios for each, at constant accelerating voltage. This necessitates changes in the initial magnetic field and electric sector voltage to be selected manually, and also results in a new mass scale. This can be time-consuming and wasteful where sample size is limited. A much faster way of generating constant parent ion spectra for all required parents is to set up on the initial precursor (P_1), at B_1, E_1 and V_1 in the normal manner, and then for each subsequent precursor P_n change the accelerating voltage to $P_n V_1/P_1$ and scan at constant B/E using the same ratio as set up for P_1 $(=B_1/E_1)$. This generates a B/E scan from each precursor without the need to change the B/E ratio, and at the same time maintains the correct mass scale for all scans.[5] This mass scale is normally already adjusted for the apparent mass of the 1st FFR daughters $(=D^2/P$ where D is the m/z value of the daughters formed in the ion source), so that ions appear at their actual m/z values.

While it is not desirable to lower accelerating voltage in general, in practice a moderate lowering of voltage results in an acceptable loss in transmission. If parents less than $0.8\,P_1$ are required, then V may need to be changed back to V_1 and a new B/E ratio entered.

A justification of the above considerations can be seen from the following. If two parents P_1 (at B_1, E_1 and V_1) and P_2 give rise to daughters D_1 and D_2 respectively, since $m/z = B^2 R^2/2V$ where R is the radius of curvature of the magnet, main beam daughters are transmitted at magnetic fields of $B_1(D_1/P_1)^{1/2}$ and $B_1(D_2/P_1)^{1/2}$, while 1st FFR daughters will be transmitted at $D_1 B_1/P_1$ and $D_2 B_1/(P_1 P_2)^{1/2}$ respectively. In the normal linked scan at constant B/E from P_1 at constant V, 1st FFR D_1 ions would require an electric sector voltage of $D_1 E_1/P_1$ and all daughters would be transmitted at a B/E ratio of B_1/E_1. Similarly, a normal linked scan at constant B/E from P_2 at V_1 would produce 1st FFR D_2 ions at $B = D_2 B_1/(P_1 P_2)^{1/2}$ and $E = D_2 E_1/P_2$, and a B/E ratio of $P_2 B_1/E_1(P_1 P_2)^{1/2}$.

The same 1st FFR ions will be transmitted at different accelerating voltage, provided the ratios E/V and B^2/V remain constant, as m/z is proportional to both. Hence, the new values are $x^{1/2}B_n$, xE_n and xV_n, where B_n, E_n and V_n are the original values for D_2, and x is the factor by which V is to be changed. If the B/E ratio is to be the same as for P_1 daughters, substitution gives

$$\frac{x^{1/2}D_2 B_1 P_2}{x(P_1 P_2)^{1/2}D_2 E_1} = B_1/E_1$$

$$x^{1/2} = P_2/(P_1 P_2)^{1/2}$$

$$x = P_2/P_1$$

The new values then become $D_2 B_1/P_1$, $D_2 E_1/P_1$ and $P_2 V_1/P_1$. Thus, all constant parent scans can be generated at the same B/E ratio. The mass scale as set up for P_1 is still correct since $D_2 B_1/P_1$ is the magnetic field required, before accelerating voltage was changed, to focus any D_2 ions arising from P_1. Similarly, main beam P_2 ions are also placed on the correct mass scale.

This procedure facilitates the rapid acquisition of all constant parent scans for a given sample, with only a few seconds required to manually adjust the accelerating voltage to the calculated positions between successive scans. A further advantage is that an ion from a reference compound such as heptacosatributylamine, slightly lower than the highest precursor required, can be used for the initial setting up of the B/E ratio, and the accelerating voltage increased to bring in P_1 before successive reduction for subsequent precursors. Thus, any valuable sample

is not required prior to the actual acquisition of the data.

There are also analytical applications for this method using selected metastable ion monitoring for the analysis of mixtures, either in conjunction with gas chromatography mass spectrometry or as direct mixture analysis.[7-9] In this case an internal standard could be employed with rapid switching between daughters from the sample and the standard. The linked scan at constant B/E would be linked to accelerating voltage as well as to a peak switching unit, thus enabling any daughter from any parent to be readily focused. Furthermore, computer acquisition of normal linked scan data would be facilitated by using the same mass scale for different constant parent scans, thus requiring at most only a few calibration files to cover the entire mass range.

Yours

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Dear Sir

Distinction Between Epimeric 3-Hydroxy Steroids by Mass Analysed Ion Kinetic Energy Spectrometry

Steroids have been extensively studied by conventional mass spectrometry,[1] and it is known that the stereochemistry of the A/B ring junction and the orientation of a hydroxy substituent at the ring system may in-

fluence the peak pattern of the mass spectra.[2] Recently, Larka et al.[3] have shown that epimers of certain steroidal hydrocarbons and ketones can be distinguished by differences in the kinetic energy released during $CH_3^{\cdot}$ loss from the molecular ions. Usually, the value of $T_{0.5}$ is larger for the 5α-isomer. During the course of an extended study of steric effects in the mass analysed ion kinetic energy (MIKE) spectra of stereoisomeric cyclic alcohols[4] we also

investigated the fragmentations of metastable molecular ions of some stereoisomeric 3-hydroxy steroids after 70 eV electron impact ionization in the 2nd field free region of a VG ZAB 2F mass spectrometer. For all the compounds investigated loss of $CH_3^{\cdot}$ and H_2O, respectively, from metastable molecular ions was observed. The values of the kinetic energy T released during these fragmentations are given in Table 1. It is clearly seen that the correlation observed by

Larka et al.[3] between $T_{0.5}$ of $CH_3^{\cdot}$ loss and the stereochemistry of the A/B ring junction does not hold for these hydroxy steroids. We suspect that the 3-hydroxy substituent favours cleavage of the A ring and that the loss of a methyl group from metastable ions occurs after this ring cleavage. This would also agree with the behaviour of metastable molecular ions of substituted cyclohexanols and decalols.[4] Similarly, no general correlation is found between $T_{0.5}$ of the H_2O elimination and the stereochemistry of the 3-hydroxy steroid (Table 1). Small differences in the $T_{0.5}$ values are observed for the 5α- and 5β-epimers, but it obviously depends on the other substituents of the 3-hydroxy steroids as to which epimer will give a larger value of T.

The MIKE spectra of the 3-hydroxy steroids contain distinct signals for ions formed by cleavage of ring D[1] in addition to signals for the loss of $CH_3^{\cdot}$ and H_2O. With the exception of the androstan-3-ol-17-ones, which give rise to an m/z 246 ion, this additional signal appears at m/z 233. As can be seen from the data given in Table 2, the intensity distribution in the MIKE spectra of the 3-hydroxy steroids depends markedly on the stereochemistry of the A/B ring junction. Ths MIKE spectra of the 3-hydroxy-5β-steroids with a cis-A/B ring junction are dominated by the signal for loss of H_2O, while the MIKE spectra of the 5α-epimers also show prominent signals for loss of $CH_3^{\cdot}$ and formation of ions by D ring cleavage. This is seen most clearly in the MIKE

spectra of the cholestan-3-ols, but also holds for the dihydroxy steroids. Obviously, the signal for $[M-H_2O]^{+\cdot}$ ions arises mainly by loss of the 3-hydroxy group. The orientation of the 3-hydroxy group at the steroid skeleton has only a small effect on the intensity of the $[M-H_2O]^{+\cdot}$ signal; however, a 3α-hydroxy substituent gives rise to somewhat larger signals for H_2O loss. The small effect of the axial or equatorial orientation of the 3-hydroxy group on the intensity of the H_2O elimination from metastable molecular ions indicates that metastable molecular ions losing H_2O and sampled in the 2nd field free region of the ZAB 2F mass spectrometer are not only intact molecular ions with a small amount of excess energy but also ions which have lost most of their ex-
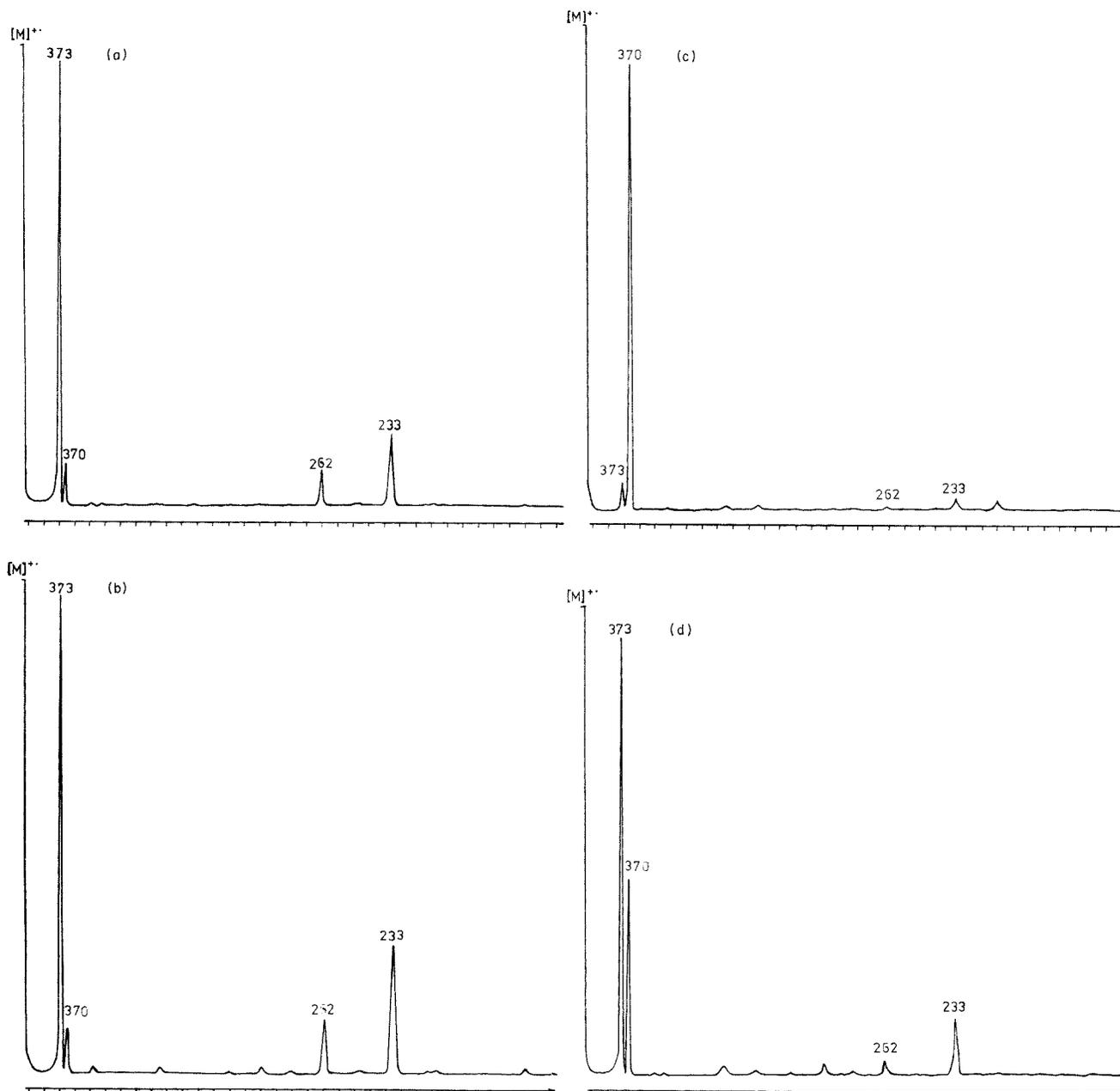

Figure 1. 70 eV MIKE spectra of the molecular ions of (a) 5α-cholestan-3α-ol, (b) 5α-cholestan-3β-ol, (c) 5β-cholestan-3α-ol, (d) 5β-cholestan-3β-ol.

Table 1. Translational energy release for the reactions $[M]^{+\cdot} - [M-CH_3]^+$ and $[M]^{+\cdot} - [M-H_2O]^{+\cdot}$ from epimeric 3-hydroxy steroids

Compound	$T_{0.5}$ $[M-CH_3]^+$	(meV)a $[M-H_2O]^{+\cdot}$
5α-Cholestan-3α-ol	61	44
5α-Cholestan-3β-ol	64	44
5β-Cholestan-3α-ol	46	47
5β-Cholestan-3β-ol	42	46
5α-Androstan-3α-ol-17-one	95	50
5α-Androstan-3α-ol-17-one	100	50
5β-Androstan-3α-ol-17-one	—	43
5β-Androstan-3β-ol-17-one	99	40
5α-Androstan-3α,17β-diol	60	38
5α-Androstan-3β,17β-diol	60	38
5β-Androstan-3α,17β-diol	—	42
5β-Androstan-3β,17β-diol	54	50
5α-pregnan-3α,20α-diol		
5α-Pregnan-3β,20α-diol	63	46
5α-Pregnan-3β,20β-diol	65	38
5β-Pregnan-3α,20α-diol	—	27
5β-Pregnan-3α,20β-diol	—	27
5β-Pregnan-3β,20α-diol	74	29

a Measured at 50% of maximum peak height and corrected for the width of the main ion beam.

Table 2. MIKE spectraa of the molecular ions of epimeric 3-hydroxy steroids

Compound	$[M-CH_3]^+$	$[M-H_2O]^{+\cdot}$	m/z 262	m/z 233	m/z 216	
5α-Cholestan-3α-ol	65	6.5	7.6	21	—	
5α-Cholestan-3β-ol	63	5	7	19	—	
5β-Cholestan-3α-ol	6	86	1	3	2	
5β-Cholestan-3β-ol	60	22	2	11	1	
	$[M-CH_3]^+$	$[M-H_2O]^{+\cdot}$	m/z 257	m/z 254	m/z 246	m/z 213
5α-Androstan-3α-ol-17-one	17	48	2	5	20	4
5α-Androstan-3β-ol-17-one	14	37	3	7	24	7
5β-Androstan-3α-ol-17-one	5	85	1	—	3	—
5β-Androstan-3β-ol-17-one	13	73	3	—	6	1
	$[M-CH_3]^+$	$[M-H_2O]^{+\cdot}$	m/z 256	m/z 248	m/z 233	m/z 166
5α-Androstan-3α,17β-diol	31	44	1	1	15	5
5α-Androstan-3β,17β-diol	31	27	—	3	28	7
5β-Androstan-3α,17β-diol	1	95	3	—	0.5	—
5β-Androstan-3β,17β-diol	8	89	—	—	1	0.5
	$[M-CH_3]^+$	$[M-H_2O]^{+\cdot}$	m/z 287	m/z 284	m/z 233	m/z 194
5α-Pregnan-3α,20α-diol	6	84	2	2	5	0.5
5α-Pregnan-3β,20α-diol	18	47	3	2	27	1
5α-Pregnan-3β,20β-diol	16	52	2	1	24	1
5β-Pregnan-3α,20α-diol	0.5	95	0.5	0.5	3	0.5
5β-Pregnan-3α,20β-diol	2	89	0.5	1	8	0.5
5β-Pregnan-3β,20α-diol	6	80	—	5	5	0.5

a 70 eV EI; only signals with intensity >0.5% total ionization are given.

cess energy by ring cleavage reactions. This agrees with the results obtained for other cyclic alcohols.[4] Nevertheless, even after cleavage of one of the C—C bonds of ring A in the molecular ions of 3-hydroxy steroids, more hydrogen atoms at the steroid skeleton are accessible for the hydroxy group by starting with a 5β-configuration and this would explain the typically large signals for $[M-H_2O]^{+\cdot}$ ions in the MIKE spectra of the 5β-epimers. The only exception to this rule observed so far is 5α-pregnan-3α,20α-diol, which gives a predominant $[M-H_2O]^{+\cdot}$ signal in spite of a 5α-configuration, probably because of the α-orientation of both hydroxy groups.

The general trend for the influence of the stereochemistry on the intensity of the $[M-H_2O]^{+\cdot}$ ions in the MIKE spectra of 3-hydroxy steroid ions has also been observed in the conventional electron impact (EI) mass spectra of these compounds.[5] How-ever, it is much more convenient to determine the stereochemistry of the A/B ring junction of a 3-hydroxy steroid by its MIKE spectrum than by the EI mass spectrum. The intensities of the $[M]^{+\cdot}$ and $[M-H_2O]^{+\cdot}$ ions in the EI mass spectra depend considerably on the type of mass spectrometer used and are very sensitive to thermal degradations of the samples in the inlet system and ion source. These effects are absent in the MIKE spectra. Furthermore, the MIKE spectra of the 3-hydroxy steroids contain only a few signals, and, as can be seen from the example shown in Fig. 1, reflect clearly the stereochemistry of the steroid skeleton.

Yours

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Dear Sir
Photoionization Mass Spectrometry in the Millisecond Range

Photoionization (PI) mass spectrometry has been a very active field of research in recent years. The high energy resolution available makes it particularly attractive for energy threshold determinations. However, its time-scale is normally limited to an ion dwell time of microseconds. Electron impact (EI) mass spectrometry has been pursued in a time-resolved mode in studies of ion/molecule reactions,[1] as well as of unimolecular decompositions of ions,[2] over a wide time range. Only a limited number of time-resolved photon impact studies has been reported.[3,4] We would like to report on a newly constructed photoionization mass spectrometer operating in a time-resolved manner from the microsecond to the millisecond range. We forsee two major applications for this type of instrumentation: (a) time-resolved appearance energy measurements for ionic fragmentations which are characterized by large 'kinetic shifts', i.e. whose rate-energy dependence is a very slowly rising function of the excess energy above threshold; (b) measurements of reaction rate coefficients for ion/molecule reactions involving ions in well-defined internal energy states.

The central part of the new instrument (Fig. 1) is a cylindrical ion trap (CIT) of the type used previously[5] in conjunction with EI experiments. The Hinteregger VUV light source is pulsed and the ions produced are trapped in the CIT. They are ejected into the quadrupole mass filter by a drawout pulse, following a variable delay time, and counted only during the ejection pulse. The characteristic dimensions of the CIT employed are $r_1 = 2$ cm and $z_1 = 1.5$ cm. Typical operating conditions are as follows: the radiofrequency potential applied to the cylindrical barrel electrode is 0.5 MHz ($\Omega/2\pi$) and 960 V peak to peak; the cylindrical electrode is biased by a +20 V DC potential. The planar end-cap electrodes are earthed and ions are ejected after a predetermined storage time by means of a −30 V pulse (width 20–50 μs) applied from