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Short Research Article

Scrambling versus specific processes in gaseous organic ions during mass spectrometric fragmentation: elucidation of mechanistic origins by isotope labelling – an overview†

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Abstract: The use of isotope labelling for the elucidation of mass spectrometric fragmentation mechanisms is demonstrated in various cases of different nature. The intermediacy of ion/neutral (I/N) complexes during fragmentation, being both analytically and fundamentally relevant, is discussed and studies on the regiospecific and irreversible intracomplex hydride transfer reactions within 'designed' deuterium-labelled I/N complexes are reported. By contrast, several types of scrambling processes, being due to reversible isomerization reactions prior to mass spectrometric fragmentation, are presented, exemplifying complete and incomplete, progressive and composite scrambling in gaseous organic ions. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: mass spectrometry; isomerization; ion/neutral complexes; H/D exchange; scrambling processes; fragmentation mechanisms

Introduction

The unimolecular fragmentation of organic ions in a mass spectrometer is often preceded by irreversible or reversible isomerization processes. In the former case, such rearrangements may open fragmentation channels which are unexpected for the given structure and even analytically irritating. This is of particular importance if the overall fragmentation involves the formation of an ion/neutral (I/N) complex, which is generated by a unimolecular process but behaves as quasi-bimolecular reaction system: the charged and the neutral fragment may undergo (quasi-intermolecular) reactions which are impossible in the original, covalently bound structure of the molecular ion.[1] The transient role of such I/N intermediates has been shown mostly by ^{2}H and sometimes also by ^{13}C labelling of the neutral precursors and pinpointed investigations of the formation and 'unimolecular' reactivity of I/N complexes based on extensive isotope labelling have been published.[2] In the case of reversible isomerization of molecular ions, isotope labelling may give rise to the formation of several isotopomeric fragment ions and, therefore, peak clusters with intensity patterns that are difficult to interpret and often simply point to the occurrence of a 'scrambling' phenomenon.[3] In turn, the mechanistic and kinetic details of such isomerization processes can be investigated by extended isotope labelling of suitable model compounds which, after ionization, allow us to study the isomerization and fragmentation sequences in greater detail. This kind of studies has led us to understand the origins of scrambling during mass spectrometric fragmentation, and different kinds of scrambling have been defined, depending on the relative rates of the competing isomerization and fragmentation processes and on the regiospecificity of the atom interchange in the reacting ions. In this article, the results of several model studies on irreversible and reversible isomerization of molecular ions prior to their mass spectrometric fragmentation are combined in order to point out the great potential of isotope labelling for the elucidation of the unimolecular reactivity of gaseous ions.

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Discussion

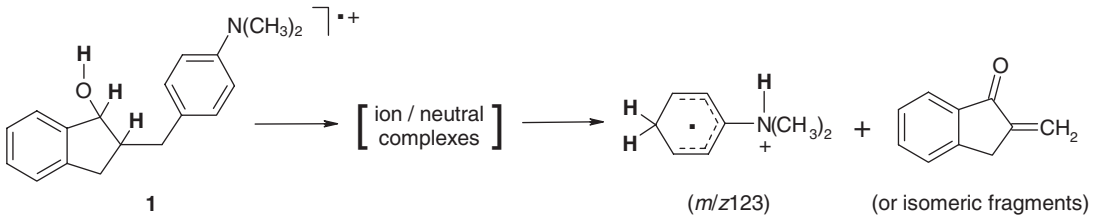
Studies on ion/neutral complexes formed during fragmentation

Among the numerous examples for the formation of transient I/N complexes,[2] the surprising elimination of neutral imines by single hydrogen rearrangement ('1H transfer') from 3,20-diaminosteroid and related radical cations belongs to the most striking ones.[1,2a] This fragmentation involves a single hydrogen transfer step between two functional groups that are mutually inaccessible in the intact molecular ions. However, within the intermediate I/N complex formed by a simple α-cleavage, an exothermic proton transfer can occur between the two mutually rotation fragments: a protonated imine protonates a neutral steroid radical at its basic, originally remote, amino group. There are much less fragmentations involving the (unidirectional) rearrangement of two hydrogen atoms ('2H transfer'), the best known being probably the so-called 'McLafferty + 1' reaction, which most likely takes place via I/N complexes, in contrast to the well-known (1H) McLafferty rearrangement. A rather peculiar unidirectional 2H transfer was found only recently to occur in ionized *N*-(ω-phenylalkyl)thiolactams: both of the hydrogen atoms of the benzylic methylene group migrate to the thiolactam functionality.[4] The intermediacy of I/N complexes to enable such 2H transfer processes has been envoked here as well.

Very rare but of potential analytical and fundamental relevance is the finding that even three hydrogens can be transferred from one moiety of a molecular ion to the other prior to the final separation of the fragments. Such unidirectional '3H rearrangement' processes were found for the first time in the molecular ions of several 3-(4-anilino)- and 3-(4-anisyl)-1-propanols, including *trans*-2-(4-dimethylaminobenzyl)-1-indanol (**1**, Scheme 1).[5] Extensive isotope labelling revealed that the two hydrogens at C-1 and C-2 and the hydroxyl hydrogen are transferred exclusively by three irreversible processes. Kinetic isotope effects point to the stereospecific role of the carbinol hydrogen (1-H).[5a] Recent studies confirmed that this fragmentation can be used for the distinction of the stereoisomeric 2-benzylcycloalcanols.[5b] While several mechanistic details of these 3H rearrangement processes have not been completely revealed, the interplay of I/N complexes is obvious to explain this unique type of fragmentation.[6]

In a reverse approach turn, the reactivity of gaseous I/N complexes can be studied in detail by synthesizing suitable model compounds which can be expected to yield, after ionization of the precursor, gaseous I/N complexes in a directed and predictive manner. Thus, I/N complexes consisting of a t-$C_4H_9^+$ ion and α,ω-diphenylalkanes have been generated from the $[M+H]^+$ ions, e.g. **2** and **3**, of various *tert*-butyl-substituted and deuterium-labelled α,ω-diphenylalkanes and related hydrocarbons, under chemical ionization (CI) conditions to study the intracomplex hydride transfer (Scheme 2).[7] This type of fragmentation was observed first in the $[M+H]^+$ ions of simpler mononuclear *tert*-butylated alkylbenzenes.[8] In our work, it was found that in all of the I/N complexes, such as the 'symmetrical' parent species [t-$C_4H_9^+$ ... $Ph(CH_2)_nPh'$] with $n = 1-10$, and many related species, the t-$C_4H_9^+$ ion abstracts a hydride from both of the benzylic methylene groups of the neutral component *with the same probability*, giving rise to the loss of isobutane.[7] For example, the mass-analysed ion kinetic energy (MIKE) spectra of protonated [1,1-D_2]- and [10,10-D_2]-labelled 1-(4-*tert*-butylphenyl)-10-phenyldecane exhibit C_4H_9D and C_4H_{10} losses in identical ratios (1:1.6), reflecting not only the regioselectivity $r = k_H^{\omega}/k_H^{\alpha} = 1.0$ but also the kinetic isotope effect operating during the hydride transfer, viz., $i = k_H/k_D = 1.6$, as an ubiquitous feature of these complexes. This 'symmetrization' of the hydride abstraction within the I/N complex indicates that the alkyl cation moves rather freely about the large neutral partner.

Symmetry is broken in congeners where an electron-releasing or electron-withdrawing substituent is introduced in one of the phenyl rings.[9] For example, the MIKE spectra of the metastable $[M+H]^+$ ions of [1,1-D_2]- and [3,3-D_2]-labelled 1-(4-*tert*-butylphenyl)-3-(4-tolyl)propane reveal that, in the corresponding complex [t-$C_4H_9^+$ ... $C_6H_5CH_2^{\alpha}CH_2CH_2^{\omega}C_6H_4CH_3$], the hydride abstraction takes place preferentially from the initially remote benzylic position, giving rise to $r = 3.3$ (with $i = 1.6$,



Scheme 1

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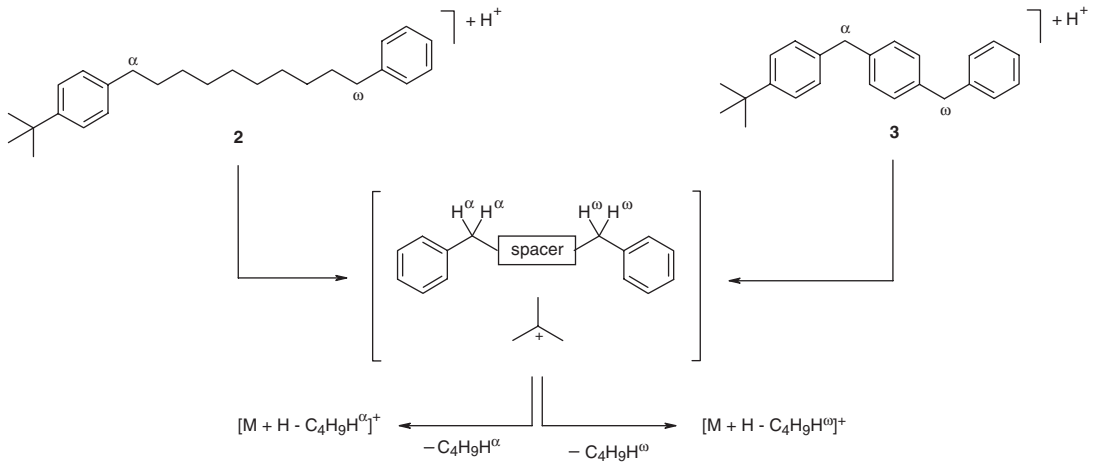

Scheme 2

again). Extensive deuterium labelling rules out all of the other positions as hydride donors, including the *para*-methyl substituent.[9a] Recent studies of various deuterium-labelled (4-*tert*-butylphenyl)-3-arylpropanes bearing methoxy, fluoro or trifluoromethyl substituents in the remote ring demonstrated that the regioselectivities of hydride abstraction span almost three orders of magnitude and that the isotope effects vary with strongly electron-releasing and electron-withdrawing substituents.[9b] Finally, congeneric I/N complexes bearing *ortho*-methyl groups were found to undergo sizeable hydride transfer from those, in competition to the benzylic α- and ω-methylene groups. The regioselectivities revealed from the fragmentation of extensively deuterium-labelled $[M + H]^+$ ions of 1-(4-*tert*-butylphenyl)-3-(2,5-dimethylphenyl)propane (**4**) are shown in Figure 1. It is intriguing that neither the *meta*-methyl group (nor the *para*-methyl group in related cases) react as hydride donors, whereas the *ortho*-methyl group in the neutral component of the I/N complex provides more than half of the overall hydride for the isobutane loss. From these findings it has been suggested that, in fact, the $t\text{-}C_4H_9^+$ ion does not move completely freely within the I/N complex; rather, it prefers to remain solvated by both of the aromatic rings of the bidentate solvent molecule.

Scrambling processes: Complete and incomplete, time-dependent (progressive) and composite intramolecular atom exchange in gaseous organic ions

In the preceding examples, no effects of atom scrambling were observed at all. Scrambling does occur, however, when an isomerization step occurring prior to fragmentation is reversible and may enable an interchange of equivalent atoms – or isotopes.[3] Hydrogen exchange processes are prevalent and have been studied in greatest detail.[10] The competition between the reverse step of the isomerization and the (forward) fragmentation and, thus, the activation energies of these processes and the ions' lifetime in the mass spectrometer determine the extent of the atom interchange prior to fragmentation. Scrambling may be *complete* ('statistical') when the activation barriers of the isomerization step(s) are relatively low. By contrast, it may become *incomplete* when the activation barriers of isomerization and fragmentation paths become similar. Moreover, since a mass spectrum is always the result of kinetically controlled reactions, the extent of scrambling generally increases with increasing lifetime of the ions in the instrument. Thus, sampling the ions after different lifetimes (e.g. in different regions of

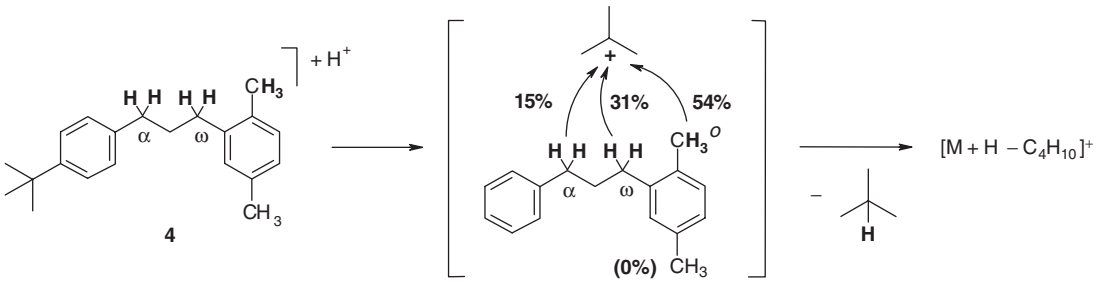

Figure 1 Regioselective hydride transfer occurring in the I/N complex formed from ions **4**.

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the mass spectrometer and/or after energetically different excitation) may reflect the kinetics of the scrambling, being a *progressive* process in time. Finally, competition between two or more isomerization reactions prior to fragmentation gives rise to *composite* scrambling behaviour. In favourable cases, complete and incomplete atom interchange processes may be observed for very same fragmentation reaction. Examples are given below.

1. *Complete scrambling*: Gaseous protonated α,ω-diphenylalkanes, $C_6H_5(CH_2)_nC_6H_6^+$ $(n = 2 - 20)$, and related hydrocarbons are known to undergo complete scrambling between the 11 protons at the aromatic rings within the microseconds' timescale.[11] Figure 2 shows the case of the $[M + H]^+$ ions of the [*ring*-D_5]-labelled isotopomer of 1-phenyl-2-(4-tolyl)ethane, **5**. The 'convex' fragment ion abundance patterns for the loss of benzene and for the loss of toluene both reveal statistical interchange of the (in this case) ten arene protons prior to fragmentation, in perfect agreement with the calculated distributions. Kinetic simulations suggest that at least ten exchange cycles (i.e. 20 transfer steps) are necessary to reach 'statistical' behaviour, which means practically 'complete scrambling'.[11] In related cases, e.g. protonated tetrabenzylmethane, at least 100 such cycles have to take place to equilibrate even 21 protons within a lifetime of ca. 1 μs.[12]

2. *Incomplete scrambling*: If the ring-to-ring proton transfer is hampered and/or the fragmentation barrier is decreased for, e.g. electronic or steric reasons, the efficiency of scrambling for ions of a given lifetime is decreased. Ironically, protonated diphenylmethane, the 'shortest' homologue among the α,ω-diphenylalkanes, represents such a case.[13] The MIKE spectrum of its [*ring*-D_5]-labelled isotopomer exhibits a 'concave' fragment ion abundance pattern for the loss of $C_6(H,D)_6$ isotopomers, reflecting incomplete scrambling of the 11 H and D atoms. The preference for the loss of the heavier benzene isotopomers clearly indicates that the ring-to-ring H^+/D^+ exchange is subject to a kinetic isotope effect. Kinetic simulations for the lifetime regime of metastable ions $[M + H]^+$ ions yielded a kinetic isotope effect, $k_H/k_D = 5$.[13] Interestingly, *intraannular* scrambling, i.e. proton exchange within the protonated rings is much faster than *interannular* proton exchange. This followed unambiguously from the identical fragmentation of two regioselectively [D_3]-labelled isotopologues, viz., protonated ([2,4,6-D_3]phenyl)- and protonated ([3,4,5-D_3]phenyl)phenylmethane.[13]

3. *Progressive scrambling*: The increase in the extent of H/D scrambling with the ions' lifetime in a mass spectrometer has been observed in numerous different cases.[3] Systematic studies concerning very short time frames (10^{-11}–10^{-9} s) have been carried out by use of field ionization kinetics.[10,14–16] On the microseconds' time scale, monitoring of the time-progressive scrambling within the ion source can be achieved by use of electron ionization (EI) at different ionization energies and by sampling the fragment ions formed from metastable ions in the field-free regions of a sector-field instrument. A particularly clear example in this context is the McLafferty reaction of ionized 2-benzylindanes, which were found to undergo a highly regioselective exchange between the *cis*-H atoms of the five-membered ring and the *ortho*-H atoms of the benzyl group.[10,17] The H/D exchange occurs via distonic molecular ions in which the hydrogen atom

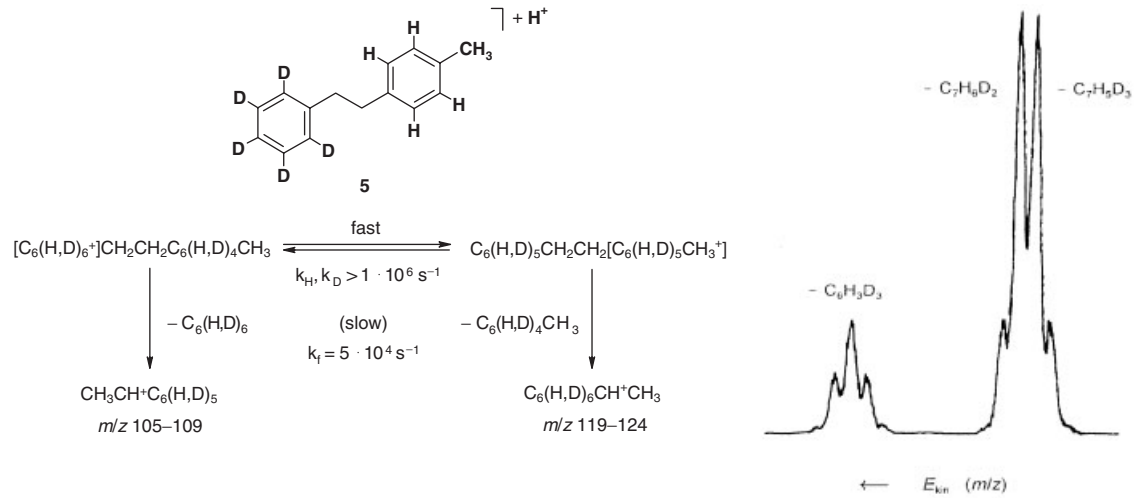

Figure 2 Interannular proton exchange in ions **5** and fragmentation by loss of benzene and toluene after complete H/D scrambling (MIKE spectrum showing convex patterns).

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transfer competes with the final C–C bond cleavage step. The observed abundance ratios of the isotopomeric fragment ions indicate that, on the average, molecular ions formed at 70 eV ionization energy undergo less than one exchange cycle within their lifetimes ($< 1\,\mu s$), whereas those formed at 12 eV (with lifetime of ca. $1\,\mu s$) undergo more than two such cycles prior to fragmentation. Metastable ions dissociating after ca. $20\,\mu s$ suffer more than seven forward and seven backward H transfer steps, giving rise to apparently complete scrambling.

4. *Composite scrambling*: Most complicated situations arise when two or more mechanistically different scrambling processes with different degrees of progression precede the same fragmentation reaction of an ion. Rare cases where three components of such composite scrambling have been identified and their relative weights estimated are protonated toluene[18–20] and protonated xylene[21a] and 6,6-dimethylfulvene.[21b] Metastable toluenium ions eliminate H_2 and CH_4, but only a 60% fraction of the ions lose a methane molecule containing the original α-C atom.[3,19] The other, 40% fraction undergoes complete C-scrambling by reversible ring expansion/contraction processes. This has been inferred from the ratio of the $^{13}CH_4$ and $^{12}CH_4$ losses from [α-^{13}C]-labelled toluenium ions and corroborated by the fragmentation of several deuterium-labelled isotopomers, e.g. **6a** and **6b** (Figure 3).[10,18] In both cases, the observed pattern for the losses of $C(H,D)_4$ can be understood as being composed of a 40% fraction of completely H/D-scrambled ions (bold bars) and a remainder fraction (60%) of incompletely scrambled ions (slim bars). Moreover, one-third of the latter fraction (20%) suffers slow, and thus incomplete, exchange between the α-H and the *ring*-H atoms, whereas two-thirds (40%) undergo truly specific loss of methane (i.e. $C^{\alpha}H_3^{\beta}H^{ring}$). Finally, a third component of scrambling, the ubiquitous fast *intraannular* H/D exchange in protonated arenes (*vide supra*) is evident from the pattern obtained from ions **6b**. Well separated from the $C(H,D)_4$ pattern reflecting the 40% fraction of ions behaving 'statistical', the lightest methane isotopomers expelled from the 60% fraction of ions are lost in the ratio $[CH_3D]:[CH_4] = 34.4:5.8$, i.e. close to what is expected for complete 5D/1H scrambling by fast proton ring walk about the benzenium ring of **6b** prior to fragmentation.

Conclusion

The reaction paths and mechanisms of mass spectrometric fragmentation reactions can be very complicated. They may give rise to unexpected and even analytically irritating peaks but, by use of extended isotope labelling and well-designed model precursors, many mechanistic details can be elaborated. As demonstrated in the present account, this holds for the studies of specific reactions in transient I/N complexes but, in particular, for various scrambling processes of different kinds that precede the unimolecular fragmentation of organic ions in the mass spectrometer.

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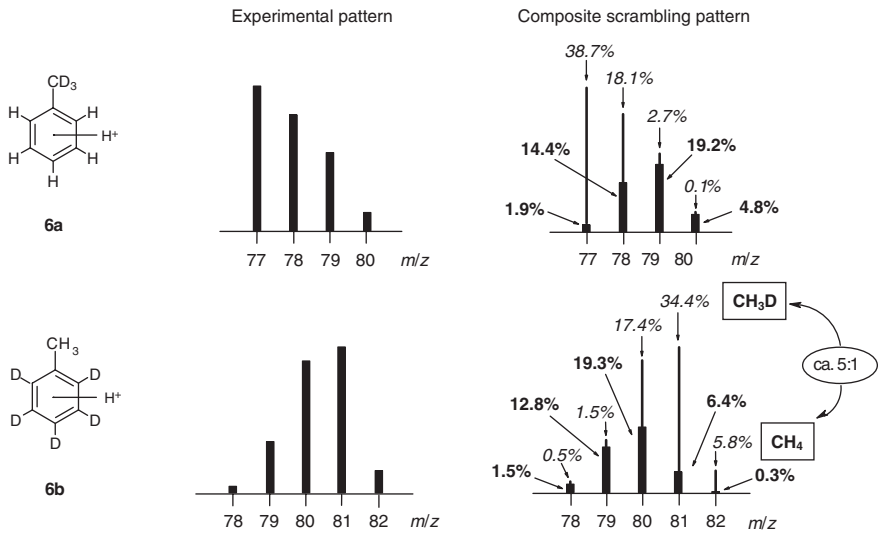

Figure 3 Experimental abundance patterns for the loss of $C(H,D)_4$ from metastable ions **6a** and **6b** and contributions from complete (bold lines) and partial scrambling (slim bars, see also text).

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