Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 1115–1123.
Published online 21 June 2007 in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1386

JLCR

Review Article

Isotopic labelling in mass spectrometry as a tool for studying reaction mechanisms of ion dissociations†

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Received 10 April 2007; Accepted 16 April 2007

Abstract: Perhaps the greatest influence that isotopic labelling experiments have had on organic mass spectrometry is that reaction mechanisms originally borrowed from the chemistry of neutral counterparts have proved to be inadequate for explaining the results. It was therefore necessary to devise completely new types of fragmentation mechanisms and unconventional structures for organic gas-phase cations. In most cases the labelling technique allows one to discover the positions at which the label atoms are found in both the charged and neutral products of an ion's dissociation. These experimental results are often difficult to rationalize by any simple mechanism, but they nearly always indicate how chemical computations should be directed in order for the latter to be able to provide a better mechanistic understanding. This short article describes some significant studies involving D and 18-O labelling that well support the above assertions, using as examples the behaviour of some quite simple organic molecules. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: ion rearrangement mechanisms; computational chemistry; tandem mass spectrometry

Introduction

The discovery of the naturally occurring isotopes of the common elements dates back over 100 years, to the earliest days of mass spectroscopy, when it was the method of choice for the fundamental studies that first measured relative isotopic masses and abundances. The history of this era is to be found in the classic book by Aston.[1]

It was only after the Second World War that mass spectrometers first became commercially available and thereafter the recording of the mass spectra of organic compounds rapidly expanded. The spectra quickly assumed a major qualitative role in compound identification and in quantitative analyses, and the ever-increasing sensitivity of the method guaranteed its appearance in all contemporary fields of trace analysis, where it reigns supreme today. Current databases that are used for general mass spectrometric analysis, comprise as many as 165 000 mass spectra.

The earliest efforts to understand the chemistry that gives rise to the mass spectra relied heavily upon analogies from condensed-phase chemistry and they were aided by deuterium labelling studies, that isotope being by far the cheapest available, when compared with ^{13}C, ^{15}N, ^{18}O, etc. Indeed, it was the increasing use of isotopic labelling in all areas of organic chemistry that led to the founding of this journal, 50 years ago. Note, however, that whatever the purpose of the study, kinetic, mechanistic or otherwise, mass spectrometry remained the most frequently chosen method to discover the fate of the label atom(s).

Deuterium labelling in mass spectrometric studies

Deuterium labelling has long been associated with the unravelling of reaction mechanisms in general chemical processes as well as with the fragmentation of organic ions in mass spectrometers. In an early book

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Contract/grant sponsor: Natural Sciences and Engineering Research Council of Canada
† Paper published as part of a special issue on 'Recent Developments in the Use of Isotopically Labelled Molecules in Chemistry and Biochemistry'.

on mass spectrometry by Biemann[2] a whole chapter is devoted to the (still considerable) difficulty of discovering by mass spectrometry, where, as the result of a synthesis or an exchange reaction, deuterium atoms are located in the labelled molecule. That and the book by Budzikiewicz *et al.*[3] describe examples of ion fragmentation mechanism studies using deuterated molecules, but the then lack of a framework based on known or established ion structures is evident, the bias being towards the use of analogies from established condensed-phase mechanisms for neutral counterparts. The discussion of mechanisms in both these books is centred on the conventional, complete (70 eV) electron ionization (EI) mass spectrum of the organic molecule.

Two reviews[4,5] that appeared in the 1970s showed *inter alia*, the increasing power of deuterium labelling experiments to uncover hidden hydrogen rearrangements, some of which were fully revealed by labelling, but only in conjunction with observations of the dissociation chemistry of metastable ions.[6] Metastable ion (MI) mass spectra uniquely permit the study of the few, lowest energy fragmentations of a mass-selected ion and so can provide very specific information as to the fate of the label.

An early example is that of ionized benzoic acid, $C_6H_5COOH^{\cdot+}$. Its EI mass spectrum contains a major ion peak corresponding to the loss of $OH^{\cdot}$, but experiments with metastable $C_6H_5COOD^{\cdot+}$ and related labelled ions showed that the carboxyl-D was equilibrated with the two *ortho*-hydrogens before fragmentation, thus explaining the 2:1 loss of OH:OD in the MI mass spectrum.[7,8] Such discoveries of hidden hydrogen rearrangements had an additional advantage in that they revealed the presence of hitherto unsuspected stable isomers of the fragmenting ion. Indeed, our present ability to uncover reaction mechanisms through the use of isotopic labels is now inseparable from the identification of most or all of the participating ion structures.

The somewhat related topic of kinetic isotope effects in mass spectrometry has recently been reviewed in an encyclopedia.[9]

By the end of the 1970s a mass spectrometer of great versatility had become available, the VG-Analytical ZAB-2F instrument.[10] This double-focusing apparatus had so-called 'reversed-geometry', with mass separation (a magnetic field, (*B*) preceding energy selection (an electric sector, *E*). In the same era, the powerful technique of collision-induced dissociation (CID) was introduced,[11] permitting the fragmentation characteristics of mass-selected ions having keV translational energy to be observed. The resulting CID mass spectra contain vital structural information and the experiments can be applied not only to ions from the ion source (i.e. those that contribute to the normal mass spectrum) but also to the charged (and neutral) fragments from metastable ions. It is particularly important to note that mass-selected ions submitted to collisional excitation are species with insufficient energy to dissociate, their internal energy distribution necessarily lying below their lowest energy dissociation threshold. Even if below the latter energy the ions can undergo a rearrangement, the majority would have retained their original structure.

A good example concerns the labelled molecular ion of 1,3-propanediol, $DOCH_2CH_2CH_2OD^{\cdot+}$, which loses CH_2O to yield a $C_2H_4D_2O^{\cdot+}$ product ion that then loses only D_2O in both its MI and CID mass spectra.[12] These observations were unique and different from all hitherto known $C_2H_6O^{\cdot+}$ isomers (e.g. ionized ethanol and dimethylether), and this new ion was assigned the structure [$^{\cdot}CH_2CH_2OD_2^{+}$]. This led to the establishing of distonic ions (odd-electron ions in which the charge and radical sites are on adjacent or separated atoms) as a very important, ubiquitous class of stable radical cations.[13]

The original ZAB instrument has been further developed into a veritable ion-chemistry laboratory[14] and the full range of experimental techniques now accessible with such mass spectrometers has been surveyed in a recent book.[6]

To summarize the present state of gas-phase ion chemistry experiments, we are now able to measure the energetics of ions and their dissociation reactions (the study of ionization and appearance energies), to investigate in detail, with the assistance of isotopic labelling, the mechanisms of their unimolecular reactions, to identify the structures of the neutral species lost in fragmentations and even to observe the chemistry of ions' neutral counterparts. The final task of constructing a complete potential energy surface for the chemistry of an organic ion can be achieved with the aid of contemporary methods in computational chemistry, methods based on *ab initio* molecular orbital theory and/or density functional theory.[6,15] These methods permit one to compute the relevant potential energy surface for an organic ion and (all, or many of) its isomers, including the transition states between them, and for all the low-energy unimolecular dissociations (metastable ions) that they may undergo. Note that these results also relate to the *reverse* ion–molecule or ion–radical reactions and so, for example, they have great relevance for upper atmosphere and interstellar processes.[16] A current example which involves both D- and ^{18}O-labelling concerns the ion–molecule reaction of the ionized dimer of CO, $(CO)_2^{\cdot+}$, with H_2O and is discussed in Section 8.

DOI: 10.1002.jlcr

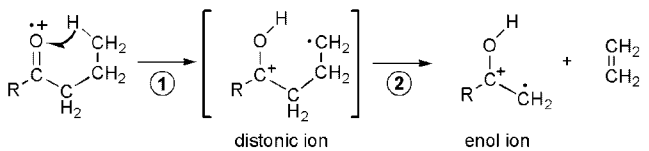
In this review we have selected some problems that illustrate the power of the above techniques, but with emphasis upon the important diagnostic role played by isotopic labelling in the unravelling of the reaction mechanisms.

The McLafferty rearrangement: is the reaction a concerted or stepwise process?

This celebrated rearrangement, known since the 1950s, provided an attractively close analogy with a well-known photochemical reaction, the so-called Norrish type II process[17] that obtains for, in particular, aldehydes, ketones and some carboxylic acids. The reaction pathway for ions is shown in Scheme 1, but for many years controversy raged as to whether the reaction was a *concerted* or a *stepwise* process. In essence then, is the intermediate ion shown in Scheme 1 a stable or only transient species?

That the reaction is indeed stepwise was nicely illustrated by a variety of labelling experiments. In the case of butyric acid, $CH_3CH_2CH_2COOH$, when the hydrogen atoms at the carbon atom γ- to the carboxyl group are replaced by D, at short reaction times, only a γ-D atom is transferred to the carbonyl group, $C_2H_2D_2$ is lost and the enol ion of acetic acid co-produced. This result could indicate a concerted process. However, for longer-lived ions, H atoms from the β-position are also transferred before the ethene is lost, showing that there must be at least one intermediate ion structure involved, to allow reversibility of the initial D-transfer.

A definitive labelling study was made by the groups of Bowie and Derrick[18] using both ^{13}C and D labels in a wide series of aliphatic and aromatic ketones. All of the ionized molecules lost ethene in a McLafferty rearrangement and showed both primary and secondary isotope effects, in keeping only with a stepwise mechanism. The McLafferty intermediate distonic ions can independently be generated from the β-cleavage of an appropriate cyclic alkanol, e.g. the distonic ion shown above is produced from the ring opening of an R-substituted cyclobutanol. It is worth noting that many of these McLafferty intermediate ions have a heat of formation that is lower than that of the ionized carbonyl compound itself.



Scheme 1

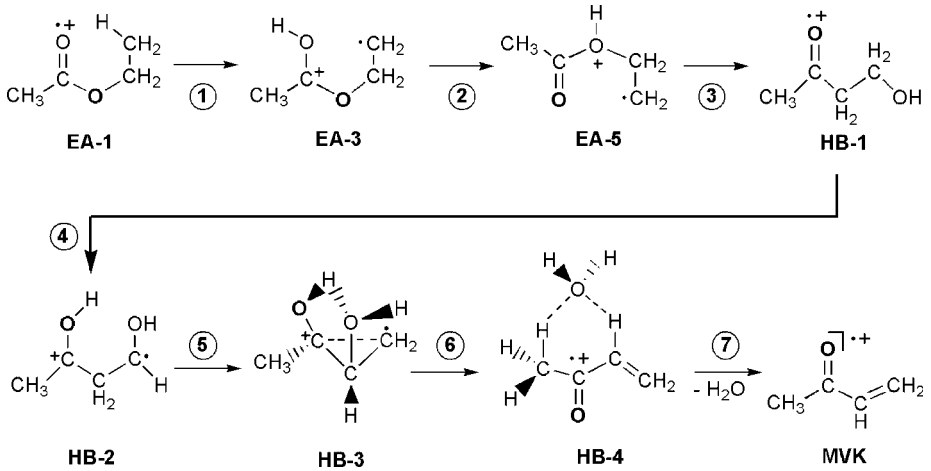
The dissociation of low-energy methylacetate ions : an unexpected neutral loss

A good example of unexpected results from deuterium labelling and the necessity of computational chemistry is shown by the complexity of the primary dissociation of the simple ionized aliphatic esters, methyl acetate and methyl propanoate. The molecular ion of the latter, $CH_3CH_2COOCH_3^{\cdot+}$, has the loss of 31 Da as its principal fragmentation. This is indeed the loss of a methoxy radical, $CH_3O^{\cdot}$, as supported by metastable $CH_3CH_2C(=O)OCD_3^{\cdot+}$ ions losing only $CD_3O^{\cdot}$ and proven by the collisional reionization mass spectrum of the neutral radical.[19] In alarming contrast, similar experiments showed that metastable $CH_3C(=O)OCH_3^{\cdot+}$ ions lose (mostly) the radical $^{\cdot}CH_2OH$, together with some $CH_3O^{\cdot}$ and that before doing so, the H/D atoms have almost completely lost their positional identity in all labelled isotopomers.[18,19] The latter result (known as 'atom scrambling') effectively vitiates any mechanistic identification. Although intermediate ion structures, including the ionized enol, $CH_2{=}C(OH)(OCH_3)^{\cdot+}$, and the distonic ion $CH_3C(OH)OCH_2^{\cdot+}$ were proposed as being involved[20,21] it remained for computational[22,23] and related[24] studies to provide a solution which indicated that the likely key intermediate ion structure that led directly to the unexpected dissociation channel was the hydrogen-bridged species,[25] $CH_3{-}C{=}O\cdots H\cdots O{=}CH_2^{\cdot+}$ (note that the 'dots' and 'dashes' between atoms in formulae represent a long bond).

The loss of H_2O from ionized ethylacetate: initially confusing labelling results

Simple esters have provided other puzzles and the case of ethyl acetate (EA) is particularly striking. The predominant dissociation of low-energy (metastable) ions is the loss of H_2O yielding ionized methyl vinyl ketone (MVK), and the same reaction applies to the enol ion, $CH_2{=}C(OH)(OC_2H_5)^{\cdot+}$. Early labelling studies provided no solution, because *both* O-atoms and *all* H-atoms are involved.[26,27]

Here then is another example where isotopic labelling fails to indicate a mechanism, but this failure nevertheless shows the way in which a computational exercise must proceed, namely it must show how the positional identity of both labels is lost prior to fragmentation. Another noteworthy feature of the above experimental study[27] was that $CH_3C(=O)CH_2CH_2OH^{\cdot+}$ the isomeric 4-hydroxy-2-butanone molecular ion (HB), lost H_2O in the μs time frame also to yield $CH_3COCH{=}CH_2^{\cdot+}$.



Scheme 2

The most recent computational study using the CBS-QB3 model and appropriate RRKM kinetic calculations[28] has resulted in the mechanism displayed in Scheme 2. It involves seven steps and can be divided into two parts.

The first part describes the isomerization of the ester ion (EA-1) into ionized 4-hydroxy-2-butanone (HB-1) via: (1) a 1,5-hydrogen shift in EA-1 yielding its distonic isomer EA-3; (2) an ethene shift in EA-3 generating another distonic ion, EA-5; (3) a 1,3-acetyl shift in EA-5 yielding a conformer of HB-1.

The second part describes the loss of H_2O from HB-1 via: (4) a 1,4-hydrogen shift in HB-1 yielding its distonic isomer HB-2; (5) a 1,2-hydroxycarbene shift in HB-2 that leads to HB-3, a one-electron long bonded species whose hydroxyl groups are bridged; (6) a formal 1,4-hydrogen shift in HB-3, in which the bridging hydrogen moves to the adjacent hydroxyl group to form the water molecule of the ion–dipole complex HB-4. This is the rate-determining step and may account for the kinetic H/D isotope effect in the water loss process. Finally, in step (7) dissociation into ionized MVK and a water molecule takes place.

Each step is a simple transformation in itself and all the consecutive steps yield a transparent picture of the apparently complex dissociation behaviour of ionized ethyl acetate. The computed energy barriers for this mechanism satisfy the energetic constraints imposed by the experimental results. It also provides a rationale for the D- and ^{18}O-labelling results, albeit indirectly.

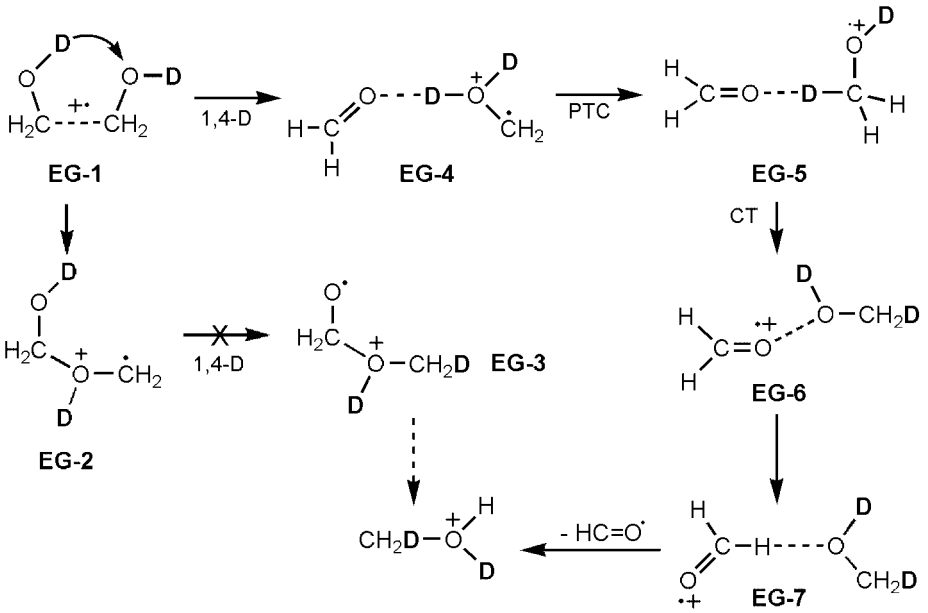
Analysis[28] of the extensive D-labelling results[27] indicates that there are two H/D equilibration reactions. In the first reaction, which is so fast that it is also observed in the normal mass spectrum, the H atoms of the ethyl chain become positionally equivalent. Reversible rearrangement of EA-1 into the distonic ion EA-3 followed by isomerization into the ion–dipole complex $CH_3C(OH)=O\cdots C_2H_4^{\cdot+}$ accounts for this exchange reaction. In the longer-lived metastable ions, the H atoms of the acetyl group also participate in the exchange reactions, almost to the statistical limit. Theory indicates that this can be rationalized by a slow but reversible isomerization of EA-3 into the enol ion EA-2, $CH_2=C(OH)OC_2H_5^{\cdot+}$. The picture that emerges is that *metastable* ethyl acetate ions consist of an (almost) equilibrated mixture of the keto, distonic, and enol isomers.

Analysis of ^{18}O-labelled ions[27] showed that for ion source decompositions and all metastable observation times, the keto and enol O atoms in both EA-1 and EA-2 are similarly preferentially lost. The rationale provided by theory[28] is that close to the threshold for water loss, EA-3 communicates with the hydrogen-bridged isomer $CH_3C(OH)O\cdots H\cdots CH=CH_2^{\cdot+}$,whose oxygen atoms become equivalent by a facile exchange of its vinyl moiety.

The loss of $HCO^{\cdot}$ from ionized ethylene glycol and acetol : crucial labelling experiments show the way

These two dissociating ions nicely illustrate the power of the isotopic labelling technique and the unexpected results that are sometimes obtained. Ionized ethylene glycol, $HOCH_2CH_2OH$, shows significant peaks at *m/z* 32 and 33 in its normal mass spectrum. The former is the well-established distonic ion $CH_2OH_2^{\cdot+}$, while *m/z* 33 is protonated methanol, $CH_3OH_2^+$. Which H-atoms are involved in the production of the latter, and wherein are they situated? For the first product, the ion $DOCH_2CH_2OD^{\cdot+}$ (EG-1) cleanly loses CH_2O to produce $CH_2OD_2^{\cdot+}$, indicating no mechanistic complexity, but

DOI: 10.1002.jlcr



Scheme 3

the atom connectivity in the protonated methanol[29,30] was found to be CH_2DOHD^+.

This result, obtained from a double collision experiment,[29] was unexpected, not least because earlier it had been proposed that the hydrogen bonding between the OH groups in the neutral persists in the gas-phase ion and thus directs its dissociation chemistry,[31] a very reasonable (and therefore highly suspect) extrapolation from neutral chemistry. The solution to this problem again relied upon the results of computations,[32] where it was shown that the (predictable) 1,4-D shift, from oxygen to carbon, in the rearranged molecular ion, EG-2, required a large activation energy, making way for the less energy demanding internal catalysis mechanism, shown above in EG-4 → EG-5, that places the hydroxyl D in the unexpected position (Scheme 3).

Recent research in gas-phase ion chemistry has revealed a surprising wealth of 'in-ion' proton transfer processes that provide a variety of low-energy paths for rearrangement processes. In the present example the mechanism can be described as 'proton transport catalysis' (PTC), with the formal ion $^{\cdot}CH_2OH_2^+/O{=}CH_2$ (EG-4), being converted to $CH_3OH^{\cdot+}/O{=}CH_2$ (EG-5). Note that solitary $CH_2OH_2^+$ and $CH_3OH^{\cdot+}$ ions do not interconvert because the barrier for the associated 1,2-H shift is prohibitively high.[33] The final steps of the reaction involve charge transfer (CT) followed by rotation of the incipient formaldehyde ion into ion EG-7, the reacting configuration for the loss of $HCO^{\cdot}$.

It is useful to comment on 'in-ion' catalyses at this juncture. This type of reaction typically takes place in a species that is, for example, a rearranged conventional molecular ion, a structure that can formally be regarded as an ion–molecule complex, or in an ion–molecule complex formed by a bimolecular encounter in the gas phase. The first of these reaction types to be identified was 'PTC'.[34] Here, the (formal) ion donates a proton to the neutral molecule yielding a free radical. The latter then is reprotonated at a different site to produce an otherwise inaccessible H-shift isomer of the original ion. This will only happen if the proton affinity of the neutral lies between those of the proton acceptor sites in the free radical.[34]

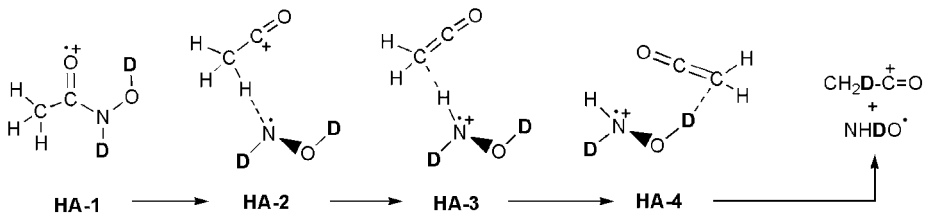
The chemistry of complexes of ionized methanol with a water molecule, generated in a high-pressure ion source[35] provides a good example; here the methanol donates a proton from the methyl group to the water molecule and receives back a proton that goes to the oxygen atom of the $^{\cdot}CH_2OH$ radical,[34b] thereby producing the more stable distonic form of ionized methanol, the ion $^{\cdot}CH_2OH_2^+$.

By a somewhat different pathway, labelled ionized acetol, $CH_3COCH_2OD^{\cdot+}$, loses $HCO^{\cdot}$ to yield CH_3CDOH^+ ions, i.e. with the hydroxyl-D at the 1-C atom, not at O, as would have been predicted by the above early 'internal H-bonding' proposal. Again the mechanism required computational chemistry for its full unravelling[36] and the pathway requires two successive proton transfers, the first from a $^+CH_2OD$ ion to the acetyl radical at the keto-carbon, and the second, a hydrogen from ionized formaldehyde to the acetaldehyde oxygen. Other vicinal diols and β-ketols display the same basic mechanistic pathway.[32,36]

DOI: 10.1002.jlcr

The dissociation of N-hydroxyacetamide ions: an example of ion-catalysis

In the case of ionized ethylene glycol, see Section 4, a key reaction step involves 'PTC'. The complementary process, whereby a (metal) ion catalyzes the transformation of a neutral, has generated considerable interest.[37] A recent example[38] involves the conversion of the radical $NHOH^{\cdot}$ to its lower-energy isomer $NH_2O^{\cdot}$. Earlier work has shown that low-energy N-hydroxyacetamide ions, $CH_3C(=O)NHOH^{\cdot +}$ do not lose $NHOH^{\cdot}$ via direct bond cleavage, but rather yield the more stable $NH_2O^{\cdot}$ species by rearrangement.[39] Analysis of the deuterium-labelled isotopomer $CH_3C(=O)NDOD^{\cdot +}$ showed that $NHDO^{\cdot}$ is lost specifically in the rearrangement reaction. This suggested that one of the hydrogen atoms of the $NHOH^{\cdot}$ moiety is exchanged with a methylic hydrogen, leading to the proposal that the neutral loss occurred via a *quid-pro-quo* type mechanism.[40,41] The proposal is shown in Scheme 4.



Scheme 4

In this proposal, HA-2 is generated from HA-1 via migration of its $NDOD^{\cdot}$ moiety towards the methyl group of the acetyl cation, forming a C···H···N-bridged species. Proton transfer from the acetyl moiety to the nitrogen atom yields HA-3, a hydroxylamine–ketene ion–molecule complex. The final transformation, HA-3 → HA-4 can be viewed as the movement of the neutral ketene molecule in HA-3 towards the hydroxylic D of the hydroxylamine ion. In this rearrangement the hydroxylic deuterium atom is transferred to the ketene molecule, transforming it into an acetyl cation. The incipient HA-4 ions may readily decompose into the observed products. The high activation barrier of $180\,kJ\,mol^{-1}$ for the unassisted transformation $NHOH^{\cdot} \rightarrow NH_2O^{\cdot}$ was reduced to a mere $30\,kJ\,mol^{-1}$ by the catalytic action of the acetyl cation. In this case too, the deuterium labelling results played a pivotal role in the elucidation of the mechanism.

The loss of DNC from ionized 4-hydroxypyridine-OD

Ionized pyridine itself loses HCN and not HNC as its principal fragmentation channel in the μs time frame.[42] It might well have been expected that ionized 4-hydroxypyridine-OD (HP-1) would do likewise, but earlier work reported the surprising observation that metastable HP-1 ions *specifically* lose DCN or DNC.[43]

In a recent experimental and computational study[44] it could be established that the product ion generated is ionized vinylketene (VK). Unfortunately the definitive experiment to establish the connectivity of the D,C,N product was inconclusive and so recourse was made to chemical computations to establish both the product identity and the mechanism for its generation. Calculations using the CBS-QB3 model chemistry showed that the key rearrangement of HP-1 ions was to undergo a 2,3 H-shift followed by a C–N ring-cleavage to yield the distonic ion HP-2. A 1–5 D-shift to N followed by cyclization and ring-opening produces HP-5, the reacting configuration for DNC loss and the production of ionized VK as the $C_4H_4O^{\cdot +}$ fragment (Scheme 5).

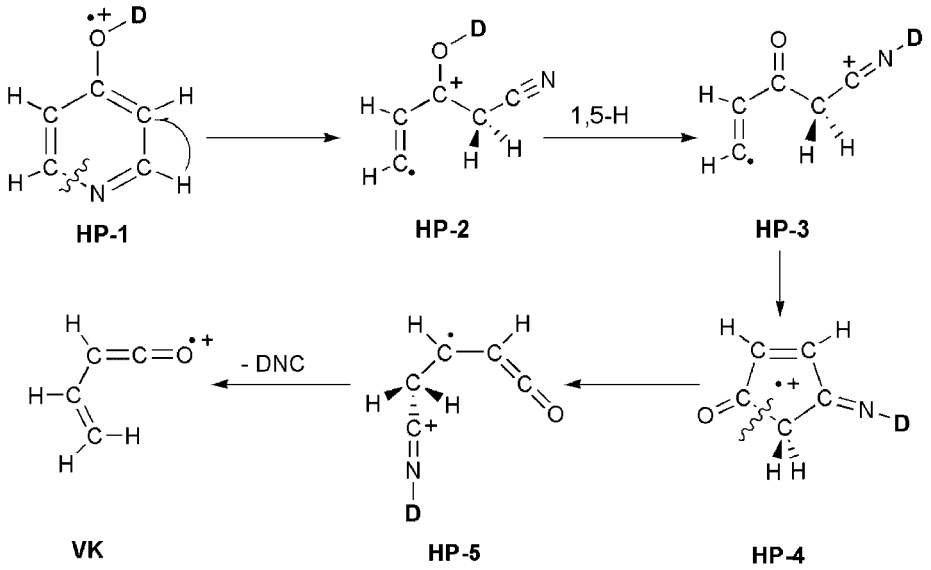
Mechanisms for the specific loss of the more stable neutral DCN were also probed but found to be too high in energy.

Oxygen-18 labelling in mass spectrometric studies

As some of the previous examples have shown, deuterium can be extremely useful as a label in mechanistic studies because H-transfers appear to play a major role in many reactions of low-energy (metastable) ions. However (often complementary), experiments with carbon-13 labelled compounds have also been frequently performed. As the early reviews[4,5,45] show, these proved to be particularly useful in probing the skeletal rearrangements of (substituted) aromatic and heterocyclic ions, including the celebrated case of the $H^{\cdot}$ loss from the toluene molecular ion. Carbon-13 (and also nitrogen-15) labelling will not be further discussed in this review: in the limited space available we will instead briefly discuss selected examples of oxygen-18 labelling.

The introduction of ^{18}O into ketones by equilibration with $H_2{}^{18}O$ in the presence of acid provides a convenient means of labelling such compounds. This technique was employed to obtain $CH_3C(=^{18}O)C(H)(^{16}OH)CH_3$, the carbonyl-oxygen-labelled acetoin molecule whose metastable molecular

DOI: 10.1002.jlcr



Scheme 5

ions dissociate by loss of an acetyl radical into a 1:1 mixture of $CH_3C(H)^{16}OH^+$ and $CH_3C(H)^{18}OH^+$ ions. This observation leaves little doubt that we are dealing with a hidden rearrangement. Indeed, when the D-labelled isotopolog $CH_3C(=^{18}O)C(H)(^{16}OD)CH_3$ was examined, it was found that the D atom in the $CH_3C(H)^{18}OH^+$ ion turns up at the carbenium carbon atom, not at the oxygen atom! The proposed mechanism for this reaction is akin to that of the $HCO^{\cdot}$ loss from ionized acetol discussed in Section 4; for a detailed discussion readers are referred to a recent review on hidden hydrogen rearrangements.[46]

The following two sections deal with studies where ^{18}O-labelling was decisive in the assignment of isomeric ion structures.

Neutral carbonic acid, $(HO)_2C{=}O$ and its ionic isomers $(HO)_2C{=}O^{\cdot+}$ and $(H_2O/CO_2)^{\cdot+}$

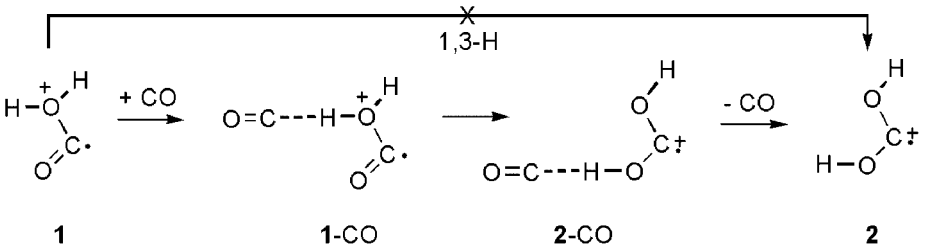
In high-school chemistry one was taught that the molecular form of carbonic acid did not exist. The emphasis was then on solution chemistry, where dissolving CO_2 in water produced only the bicarbonate anion HCO_3^- and a proton. However, an ion at m/z 62, of formula $[H_2,C,O_3]^{\cdot+}$ was readily generated by the thermal dissociation of NH_4HCO_3 in a heated mass spectrometer ion source probe.[47] The ion's CID mass spectrum was very closely similar to its neutralization–reionization mass spectrum (NRMS[6]) confirming the connectivity of the ion and the neutral as that of carbonic acid. The salient peaks were at m/z (62), 45, 44, 29, 28 and 18. In a related study,[48] the $[H_2,C,O_3]^{\cdot+}$ ion produced in a high-pressure ion source containing a mixture of CO_2 and H_2O, was shown to be the cluster ion $(H_2O/CO_2)^{\cdot+}$ in which the components retained their atom connectivities. This was shown by the use of $H_2{}^{18}O$ as reactant; the NRMS displayed no recovery signal at m/z 64, only m/z 44 $(CO_2)^{\cdot+}$, m/z 45 $(HOC{=}O)^+$ and m/z 20 $(H_2{}^{18}O)^{\cdot+}$, results in keeping with the connectivity $[H{-}O{-}H\cdots O{=}C{=}O]^{\cdot+}$, a hydrogen-bridged species found to be stable by theory.[48] Note that the labelling result shows that the cluster ion does not communicate with ionized carbonic acid.

The isomerization of $H_2O{-}C{=}O^{\cdot+}$ into $HO{-}C{-}OH^{\cdot+}$: proton-transport catalysis monitored by ^{18}O-labelling

Early mass spectrometric studies using a high-pressure ion source under chemical or photon ionization conditions[49] have shown that CO_2 containing a small quantity of CO efficiently produces the dimer ion $(CO)_2^{\cdot+}$ and that trace amounts of water readily yield $CH_2O_2^{\cdot+}$ ions. These are generated via the reaction $(CO)_2^{\cdot+} + H_2O \rightarrow CH_2O_2^{\cdot+} + CO$.

A recent study[50] addresses the identity of the $CH_2O_2^{\cdot+}$ ion structure from the above reaction and proposes that it may also play a role in the rich ion-neutral chemistry of the lower Martian ionosphere. The calculations of this study predict that the interaction of H_2O with a C(=O) moiety of the $(CO)_2^{\cdot+}$ dimer ion $O{=}C{=}C{=}O^{\cdot+}$ is an exothermic process that yields the distonic $CH_2O_2^{\cdot+}$ isomer $H_2O{-}C{=}O^{\cdot+}$ (**1**)+CO. Solitary ions $H_2O{-}C{=}O^{\cdot+}$ are predicted to retain their structure identity and to display a CID mass spectrum featuring peaks of comparable intensity at m/z 18

DOI: 10.1002.jlcr



Scheme 6

($H_2O^{\cdot+}$) and *m/z* 29 (HCO^+). However, the ion was generated under high-pressure conditions and theory then predicts that a CO (but not CO_2) catalyzed isomerization takes place into HO–C–$OH^{\cdot+}$ (**2**), which is more stable than its conventional isomer, ionized formic acid, HC(=O)$OH^{\cdot+}$ (**3**). The ensuing PTC is depicted in Scheme 6.

The incipient distonic ion (**1**) combines with a CO molecule to yield the hydrogen-bridged ion **1**-CO. The key step in the reaction is the CO-assisted 1,3-H shift yielding ion **2**-CO whose decarbonylation yields HO–C–$OH^{\cdot+}$, ionized dihydroxycarbene. The experiments of this study are in concert with theory. The ion generated under standard chemical ionization conditions clearly is HO–C–$OH^{\cdot+}$ as witnessed by its characteristic CID mass spectrum. However, when the pressure was lowered and a trace of $H_2{}^{18}O$ was used as the reactant, a CID mass spectrum was obtained entirely compatible with the presence of HO–C–$^{18}OH^{\cdot+}$ in admixture with $H_2{}^{18}O$–C=$O^{\cdot+}$; the latter ion dissociates into $H_2{}^{18}O^{\cdot+}$ and $HC^{16}O^+$ without O-atom equilibration, as predicted by theory. These results support the formation of **2** via the isomerization **1**+CO → **2**+CO: at low pressure, stabilized encounter complexes with CO are less readily formed so that part of the incipient ions **1** retain their structure identity.

Conclusion

In this short review we have emphasized the continuing key role played by isotopic labelling experiments, even though at first glance the observations may sometimes appear to be undecipherable. However, the advent of contemporary methods in computational chemistry has resulted in highly satisfying solutions to the apparently intractable mechanistic dilemmas. Thus in concert, the two approaches now comprise an essential tool for the understanding of gas-phase ion chemistry.

Acknowledgements

J. K. T. thanks the Natural Sciences and Engineering Research Council of Canada (NSERC) for financial support.

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DOI: 10.1002.jlcr