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Dissociative Protonation Sites: Reactive Centers in Protonated Molecules Leading to Fragmentation in Mass Spectrometry

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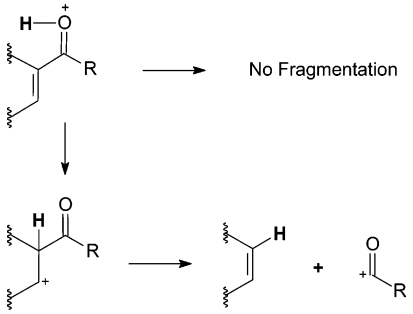

It is often found in mass spectrometry that when a molecule is protonated at the thermodynamically most favorable site, no fragmentation occurs, but a major reaction is observed when the proton migrates to a different position. For benzophenones, acetophenones, and dibenzyl ether, which are all preferentially protonated at the oxygen, deacylation or dealkylation was observed in the collision-induced dissociation of the protonated molecules. For para-monosubstituted benzophenones, electron-withdrawing substituents favor the formation of $RC_6H_4CO^+$ (R = substituent), whereas electron-releasing groups favor the competing reaction leading to $C_6H_5CO^+$. The $\ln[(RC_6H_4CO^+)/(C_6H_5CO^+)]$ values are well-correlated with the σ_p^+ substituent constants. In the fragmentation of protonated acetophenones, deacetylation proceeds to give an intermediate proton-bound dimeric complex of ketene and benzene. The distribution of the product ions was found to depend on the proton affinities of ketene and substituted benzenes, and the kinetic method was applied in identifying the reaction intermediate. Protonated dibenzyl ether loses formaldehyde upon dealkylation, via an ion−neutral complex of the benzyloxymethyl cation and neutral benzene. These gas-phase retro-Friedel−Crafts reactions occurred as a result of the attack of the proton at the carbon atom to which the carbonyl or the methylene group is attached on the aromatic ring, which is described as the dissociative protonation site.

Introduction

Protonation is an elementary reaction involved in proton transfer[1] and hydrogen bonding[2] in various chemical and biological systems. Over the past four decades, a tremendous amount of effort has been devoted to the determination of the site of protonation for molecules from those as small as carbon monoxide[3] to large ones such as peptides and proteins.[4] For multifunctional molecules, protonation may occur at different sites resulting in different forms of protonated molecules (MH^+). Binding of a proton at the thermodynamically most favorable site gives rise to the most stable MH^+ ions from which the

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intrinsic physicochemical properties of molecules, e.g., the proton affinity (PA),[5] can be measured in the gas phase by mass spectrometry.[6] Under given conditions, the site of protonation can be determined by using various techniques, such as UV, NMR, and mass spectrometry. However, it has been shown that in some cases a molecule may have a different "preferred" site of protonation in solutions[7] than in the gas phase when the solvent effect is involved.[8] It is also interesting that when studied by different methods of mass spectrometry, a molecule can be found to be protonated at sites that are not energetically favored,[9] reflecting the thermodynamically vs kinetically controlled nature of the protonation process.[10]

Mass spectrometry plays an important role in the fundamental studies of protonation of molecules in the gas phase. On the other hand, protonation is also important in the application of mass spectrometry to structural elucidation, since the molecules of interest are often ionized by protonation to offer structural information. To begin interpretation of the fragmentation of a protonated molecule, one might first want to tackle where the proton goes in the molecule because it is common that cleavages are directed by the charge brought in by protonation.[11] In either the traditional chemical ionization or the contemporary electrospray ionization mass spectrometry (ESI-MS), the MH^+ ion population is often predominated by one structure in which the external proton is attached to a specific position of the molecule. However, the major fragmentation reaction may *not* take place from this dominant structure unless the ionizing proton migrates to a different position. As a typical example, protonation of the amide has been repeatedly shown to occur at the carbonyl oxygen.[12] In contrast, the major fragmentation of the MH^+ ions of amides, i.e., loss of the amine (or ammonia), takes place *only* when the amide nitrogen is protonated, even though the *N*-protonated species is 14.3 kcal/mol higher in energy than the *O*-protonated isomer, in the case of formamide, and there is a high energy barrier between the two structures.[13] This has led to the proposal of a "mobile proton model",[11,14−16] which describes the mobility of the proton *across* the MH^+ ion, and is now widely used in the mass spectrometry of peptides and proteins.

The amide is not only the elementary unit of peptides but also an important functional group of many drug molecules. Penicillins, for example, contain two amide bonds. In ESI-MS, fragmentation of the MH^+ ions of penicillins is dominated by cleavage of the β-lactam bond,[17] which requires protonation at the lactam nitrogen. For the α,β-unsaturated amides, another fragmentation reaction prevails that requires protonation at the α-carbon atom. Atorvastatin, a new drug for the treatment of high serum cholesterol, has a phenylamido moiety conjugated with the central pyrrole ring. In the collision-induced dissociation (CID) mass spectrum of its MH^+ ion (*m*/*z* 559), the base peak is observed[18] at *m*/*z* 440, corresponding to loss of phenyl isocyanate ($O{=}C{=}NC_6H_5$). By using a series of benzamides, which is similar to atorvastatin in view of the α,β-unsaturated amide moiety, we have confirmed[19] that loss of isocyanates requires a configuration where the α-carbon is protonated. Benzamides are known to be protonated preferentially at the carbonyl oxygen.[20] Since the proton affinity of a simple amide is at least 17 kcal/mol higher than that of benzene,[5] isomerization of the *O*-protonated MH^+ ion to the C_α-protonated species is an endothermic process.[21] Nevertheless, this proton transfer still takes place prior to loss of the isocyanate. We are interested in characterizing these "hot spots", i.e., positions similar to the amide nitrogen in the cleavage of the amide bond or the α-carbon of an α,β-unsaturated amide in the loss of an isocyanate, which we would like to describe as the dissociative protonation sites. Protonation at these sites results in *reactive* configurations from which a fragmentation reaction takes place without further isomerization. They are *not* necessarily the most basic sites but truly crevices (reactive centers) in the MH^+ ions leading to fragmentation in mass spectrometry.

The mobile proton model[11,14−16] is applicable not only to the flexible peptides but also to rigid molecules. As a matter of fact, the mobile feature of the ionizing proton was first observed and unequivocally characterized by Grutzmacher and coworkers.[22] In many aromatic molecules, such as the terphenyls, with a carbonyl group on one end and a methoxymethyl on the other, they made the carbonyl oxygen protonated unambiguously (by loss of an alkyl from a tertiary alcohol precursor in electron impact ionization) and observed that the proton migrates from the carbonyl oxygen to the methoxy oxygen, leading to loss of

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(21) The PA values of formamide and benzene are 196.5 and 179.3 kcal/mol, respectively. An electron-withdrawing group decreases the PA of benzene further, especially at the *ipso*-position.

methanol.[22f] Clearly, a fragmentation reaction occurs upon the arrival of the proton at a "dissociative" position. In this paper we describe the mass spectrometry of compounds for which major fragmentation reactions are observed only when the proton migrates from the thermodynamically favored position to a nonfavored position. In such a reaction, the moiety of the molecule that retains the ionizing proton could be either lost as the neutral species or found as the fragment ion. It is demonstrated that the dissociative protonation sites of the MH^+ ions are the reactive centers for fragmentation in mass spectrometry.

Experimental Section

An API-US hybrid quadrupole/time-of-flight (Q-TOF) mass spectrometer with a Z-spray source (Waters/Micromass, Manchester, UK) and a Sciex API 4000 triple quadrupole mass spectrometer with an orthogonal turbo V-spray source (MDS Sciex, Toronto, Ontario, Canada) were used. Both instruments were operated at a source temperature of 300 °C and an electrospray voltage of 3−4 kV. On the Q-TOF instrument, nitrogen was used as the desolvating and nebulizing gases, and argon as the collision gas, which was adjusted to result in a pressure of 5×10^{-3} Pa on the analyzer Penning gauge unless otherwise indicated. On the Sciex instrument, zero air was used as the desolvating gas, and nitrogen served as both the curtain gas and the collision gas. The pressure in the collision cell was 4×10^{-3} Pa as indicated by an ion gauge attached to the cell.

Benzophenones, acetophenones, and the dibenzyl ether were all commercial products, and were used without further purification. The compounds were dissolved in acetonitrile first and diluted with water containing 0.1% formic acid to a final concentration of ~1 μg/mL. The solutions were infused for electrospray ionization with a syringe pump at a flow rate of 2 μL/min. Data reported here were taken from the combined spectra acquired over a few minutes for all compounds.

Results and Discussion

The compounds selected in this study are expected to have no or only minor fragmentation *directly* from the MH^+ ions in which the proton is attached to the thermodynamically favored site; a major fragmentation reaction occurs when the proton migrates to the key dissociative protonation site. The reactions are categorized to show that the moiety of the molecule that retains the external proton may be (1) lost as the neutral species, (2) observed as the fragment ion, or (3) either the neutral or the ionic product in a dual mode. The compounds in the three groups are benzophenones, the dibenzyl ether, and acetophenones, respectively.

As the Neutral Species. In this group, the ionizing proton and the moiety of the molecule retaining the proton are eliminated as the neutral species in the fragmentation. For the unsubstituted benzophenone, the CID mass spectrum of the MH^+ ion (*m*/*z* 183) showed only one product ion at *m*/*z* 105, which is the phenylcarbonyl cation. For monosubstituted benzophenones, generally two competing product ions, $C_6H_5CO^+$ and $RC_6H_4CO^+$ (where R is the substituent), were observed in the CID mass spectra of their MH^+ ions. What is interesting is when R is a strong electron-withdrawing group (e.g., *p*-NO_2), the intensity of the $RC_6H_4CO^+$ ion is significantly *higher* than that of the $C_6H_5CO^+$ ion; whereas when R is a strong electron-donating group (e.g., *p*-$N(CH_3)_2$), the intensity of the $RC_6H_4CO^+$ ion is considerably *lower* than that of the $C_6H_5CO^+$ ion. Obviously, the stability of the product ion is *not* the key factor governing the reaction.

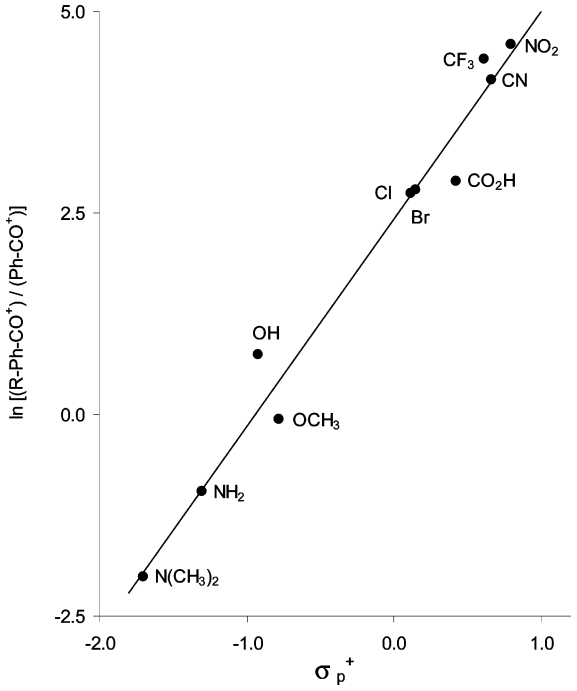

FIGURE 1. Plot of ln[(R-Ph-CO^+)/(Ph-CO^+)] vs the σ_p^+ substituent constants for the collision-induced fragmentation of the MH^+ ions of benzophenones monosubstituted at the para position. Collision energy $E_{cm} = 2.5$ eV (argon).

The reaction must be triggered by protonation at the α-carbon to which the carbonyl is attached, resulting in loss of a neutral benzene that captured the added proton. However, in view of the protonation site, the unsubstitituted benzophenone would be similar to benzaldehyde for which Kebarle[23] and Harrison[24] have concluded that the carbonyl oxygen is the preferred site for protonation. Therefore, for benzophenones, a proton transfer from the oxygen to the carbon is required prior to the fragmentation, and the reaction mechanism could be further explored by studying the substituent effects on the distribution of the product ions. For various benzophenones monosubstituted on the para position, the variation of the intensities of $C_6H_5CO^+$ and $RC_6H_4CO^+$ was well correlated with the nature of the sustituents. A plot of the intensity ratios of these two ions vs the substituent constants,[25] σ_p^+, was obtained as shown in Figure 1. Generally, all electron-attracting groups ($\sigma_p^+ > 0$) favor the formation of the $RC_6H_4CO^+$ ion, and electron-releasing groups ($\sigma_p^+ < 0$) favor the other channel to form $C_6H_5CO^+$ instead.

In view of the proton transfer, as shown in Scheme 1, from the carbonyl oxygen, the proton can migrate to the α-carbon of either ring through transition states TS1 and TS2 to generate

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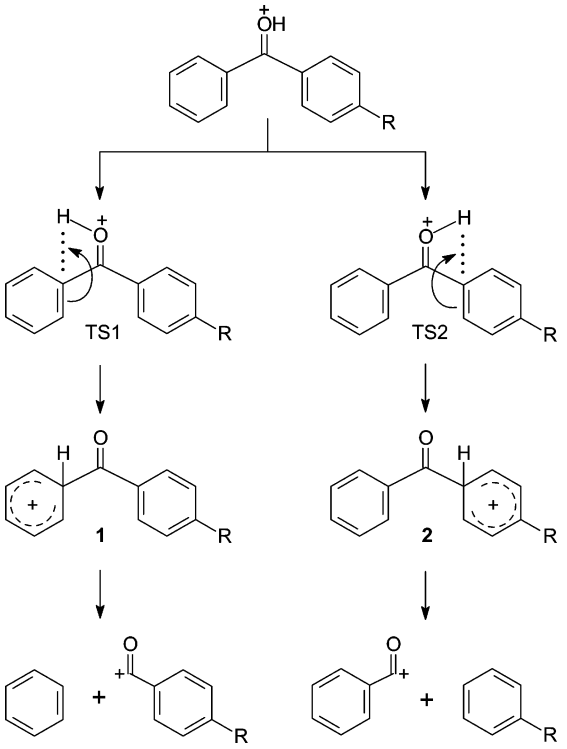
SCHEME 1



two C_α-protonated ions, **1** and **2**. The subsequent simple cleavage of the C_α−CO bond leads to the final phenylcarbonyl cations. By this mechanism, briefly, if R is an electron-withdrawing group, the C_α site in TS1 is *more* capable of accepting the proton than that in TS2 due to the destabilizing effect of the substituent, and the reaction through TS1 is more favorable. In contrast, when R is an electron-donating group, the local PA[26] at the C_α site in TS2 (para to the R group) is *higher* than that at the corresponding position in TS1, and the reaction through TS2 is favored. This is really similar to the scenario of comparing the reactivity of RC_6H_5 (R = NO_2, $N(CH_3)_2$, for example) in electrophilic substitution on the para position. The reaction is actually governed by the energy of the transition states.

To further understand the energetics of the reaction, the thermochemical data[5,27] of the fragmentation products for methoxybenzophenone are presented in Table 1. The Δ_fH value of *p*-$CH_3OC_6H_4CO^+$ is estimated to be 115 kcal/mol, assuming that the increment from R = H to CH_3O in $RC_6H_4CO^+$ is the same as that in $RC_6H_4CH{=}OH^+$ (the MH^+ ions of benzaldehydes).[27] However, the thermochemistry leads to a conjecture that formation of *p*-$CH_3OC_6H_4CO^+$ (*m*/*z* 135) is more favorable, by 17 kcal/mol, than the formation of $C_6H_5CO^+$ (*m*/*z* 105). In fact, the observed intensity of *m*/*z* 135 is even *lower* than that of *m*/*z* 105, and the $\ln(135^+/105^+)$ value is negative as shown in Figure 1 for the CH_3O substituent. We believe that the 1,3-H transfer described in Scheme 1 has a significant energy barrier for *both* channels to make them competitive. On a double-focusing magnetic sector mass spectrometer, Sun and Grutzmacher[28] studied the fragmentation of the benzophenones; in particular, they found that loss of benzene from the MH^+ ion of the unsubstituted benzophenone gives rise to a broad peak, with a kinetic energy release of 192 meV, which is much larger than a simple bond cleavage. The kinetic energy release[29] is indicative of the existence of a reverse activation energy created by a large energy barrier. Therefore, based on the mass spectral data, along with the thermochemical and the kinetic energy release information, an illustrative potential energy profile (Figure 2) is created for the reactions of methoxybenzophenone.

The proton transfer involved in the reaction is highly endothermic. The PA is 210.9 kcal/mol for benzophenone (on the carbonyl oxygen, and that would be higher with a methoxy substituent) and 179.3 kcal/mol for benzene (which should be lower at the C_α position when substituted with a carbonyl

TABLE 1. Thermochemical Data for the Species Generated in the Fragmentation of Protonated Methoxybenzophenone, Dibenzyl Ether, and Acetophenone (kcal/mol)

species	PA	$\Delta_fH°$	ref/note
CH_2O	170.4	−27.7	5, 27
CH_2CO	197.3	−11.4	5, 27
CH_3CO^+		156	27
C_6H_6	179.3	19.8	5
$C_6H_6{\cdot}H^+$		206	5, 27[a]
$C_6H_5CO^+$		168	27
$C_6H_5CH_2^+$		215	27
$C_6H_5OCH_3$		16.2	27
$(C_6H_5)_2CH_2$	191.7	39.4	5, 27
$(C_6H_5)_2CH_2{\cdot}H^+$		213	5, 27[a]
$C_6H_5CH_2OH$	192.4	−22.6	5, 27
p-$CH_3OC_6H_4CO^+$		115	estimated[b]

[a] Calculated from the heat of formation and PA of the neutral molecule. [b] Estimated from that of $C_6H_5CO^+$ by using an increment of −53 kcal/mol, see the text.

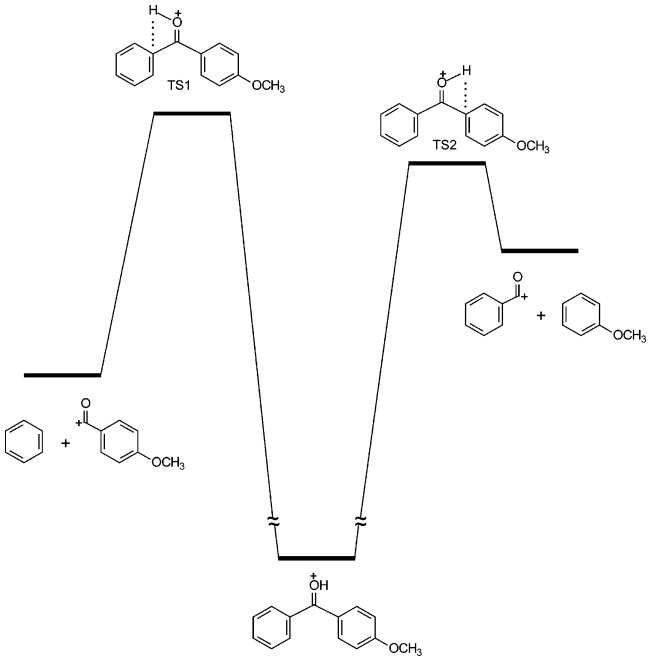


FIGURE 2. An illustrative potential energy profile for the fragmentation of the MH^+ ion of *p*-methoxybenzophenone.

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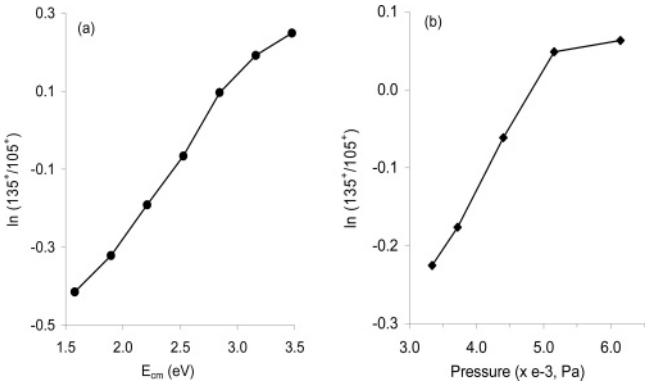

FIGURE 3. Dependence of the product ion intensities on (a) collision energy and (b) collision gas (argon) pressure in CID reactions of *p*-methoxybenzophenone.

group). Furthermore, the proton transfer from O to C_α in this reaction, also a 1,3-H shift, is similar to that from O to N in protonated amides where the existence of a significant energy barrier is proven.[13] Although an alternative 1,4-H migration to the β-position followed by a low-energy 1,2-hydride transfer[30] (the hydrogen ring-walk[26b]) might be more plausible in terms of energy requirement, that pathway would cause hydrogen scrambling. With isotope labeling in benzophenone, e.g., $C_6H_5C(=OD^+)C_6H_5$ and $C_6H_5C(=OH^+)C_6D_5$, Sun and Grutzmacher[28] have shown that only minor H/D exchange occurred in the fragmentation. Therefore, the direct 1,3-H shift is the dominant pathway.

The reactions from ions **1** and **2** to the corresponding phenylcarbonyl cations (Scheme 1) are simple bond cleavages, which do not involve major reverse activation energies. Therefore, ions **1** and **2** should reside in very shallow wells on the potential energy diagram (not shown in Figure 2). Alternatively, the proton transfer from O to C_α and the C_α−CO bond cleavage may take place simultaneously in a concerted fashion.[31] High-level theoretical calculations would be required to provide a definitive description of the reaction mechanisms in detail. Nevertheless, it is clear that the fragmentations are triggered by protonation on the C_α atoms of the phenyl groups.

It should be noted that the unsubstituted benzophenone (R = H, not shown in Figure 1) is far off the curve because the $C_6H_5CO^+$ cation from one side or the other is the same ion and the logarithmic value of the intensity ratio used in the plot is always 0 (σ_p^+ for H is also 0). For the monosubstituted benzophenones, however, the prominence of a fragmentation channel varies as the experimental conditions change. In the case of methoxybenzophenone, for example, as the collision energy decreases, the relative intensity of $CH_3OC_6H_4CO^+$ (*m*/*z* 135) decreases whereas that of $C_6H_5CO^+$ (*m*/*z* 105) increases, resulting in descending $135^+/105^+$ ratios. In addition, at a fixed collision energy, the $135^+/105^+$ ratio also decreases as the pressure in the collision cell goes down. As shown in Figure 3, the $135^+/105^+$ ratio could be much lower when extrapolated to where the effects of collision energy and collision gas pressure are negligible. In fact, the $135^+/105^+$ ratio in the metastable ion spectrum was reported[28] to be only 20:100. By using this value the curve in Figure 1 could shift downward so much that R = H would nicely fit in. More importantly, the dependence of the ion intensities on the collision energy and on the collision gas pressure, as shown in Figure 3, also indicates that the reaction leading to the *m*/*z* 135 ion requires higher activation energy than the other channel (*m*/*z* 105), consistent with the proposed potential energy profile (Figure 2).

In the Product Ion. Protonated molecules in this category dissociate so that the ionizing proton and the moiety that captures the proton remain in the product ion. In routine analysis we often found that compounds containing an allyl ether (CH═CH−CH_2OR) group may lose formaldehyde, which is an interesting fragmentation reaction that involves rearrangement. Dibenzyl ether may be viewed as one of those compounds although its methylene is conjugated with an aromatic ring rather than with a simple olefin. The CID mass spectrum of the MH^+ ion of dibenzyl ether is shown in Figure 4. Note that the collision energy is very low (E_{cm} = 0.3 eV), indicating that the molecular ion is rather "fragile". The base peak at *m*/*z* 169 corresponds to loss of formaldehyde, with an error in accurate mass measurement of only 1.8 ppm. In light of the fact that the PA values of ethers are usually higher than those of benzenes, it should be acceptable to assume that dibenzyl ether is preferably protonated on the oxygen. Protonation at the oxygen induces an α-cleavage to form the benzyl cation (*m*/*z* 91) with loss of a neutral benzyl alcohol (therefore, the oxygen is obviously also a dissociative protonation site). However, we are more interested in the major fragmentation, the loss of formaldehyde. Other reactions, including water loss,[32] are not discussed here.

In the elimination of formaldehyde, the Ph−CH_2 bond is first ruptured while the CH_2−O bond is kept intact. This certainly requires proton transfer from O to the C_α-position, as shown in Scheme 2, to form an ion−neutral complex, **3**. In mass spectrometry, it is well-documented in the past two decades that fragmentation in the gas phase may take place by way of an ion−neutral complex intermediate.[33−39] Upon loss of formaldehyde, the resulting benzyl cation attacks the neutral partner (benzene) to give the *m*/*z* 169 ion. When this ion was selected for CID, it decomposed to form the benzyl cation (*m*/*z* 91) easily, which is expected for protonated diphenylmethane. The ion−neutral complex **3** can also simply separate to eliminate the neutral benzene. The resulting benzyloxymethyl cation (*m*/*z* 121), which is evidence in support of the mechanism, is observed (Figure 4).

In the chemical ionization mass spectrum of dibenzyl ether, Shannon et al.[32] also observed the same fragmentation. In the reaction mechanism they proposed, formation of the C_β−C_β bond between the two phenyl rings might be too difficult; however, it is agreeable that the reaction is initiated by protonation at the C_α-position. Compelling evidence for the C_α-protonation was obtained from isotope labeling.[32] With the methylene on one side labeled, the MH^+ ions of *p*-RC_6H_4-CH_2-OCD_2-C_6H_5 lose both CH_2O and CD_2O, and the ratios of CH_2O over CD_2O losses were 0.8, 4, and 99 for R = Br, CH_3, and

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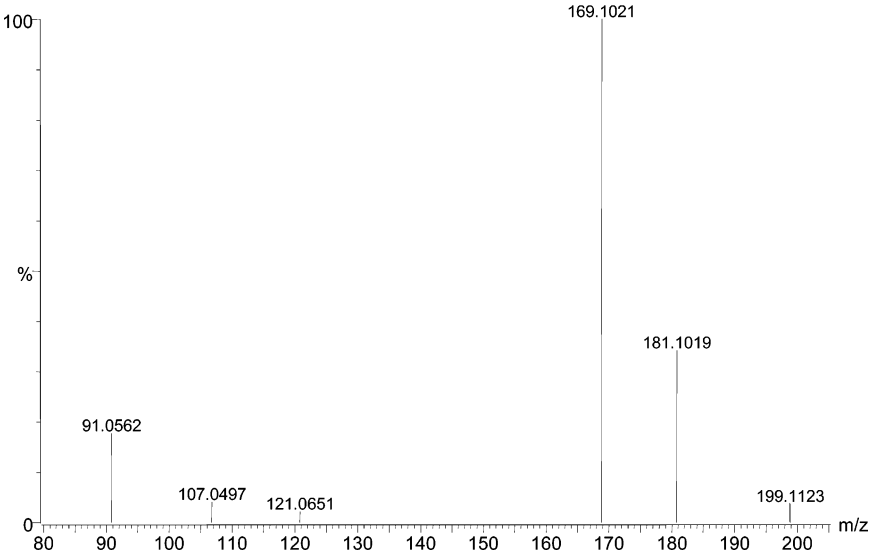
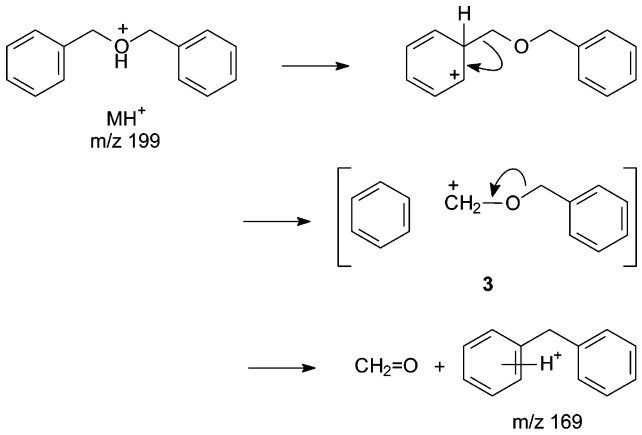


FIGURE 4. CID mass spectrum of the MH^+ ion (*m*/*z*199) of dibenzyl ether at $E_{cm} = 0.3$ eV acquired on the Q-TOF mass spectrometer with the exact mass measured for each ion.

SCHEME 2



CH_3O, respectively.[32] This strongly suggests that an electron-donating group on the ring bearing C**H**$_2$O enhances C_α-protonation on this ring (see Scheme 2) to facilitate loss of the nonlabeled formaldehyde. The substituent effect on this reaction is similar to that observed in the fragmentation of benzophenones described earlier.

When dibenzyl ether is initially protonated at the oxygen, a simple bond cleavage occurs to form the benzyl cation and the neutral benzyl alcohol; upon transfer of the proton to the C_α-position, loss of formaldehyde takes place. With the thermochemical data for the related species given in Table 1, it is found that loss of formaldehyde is overall more favorable in energy. We noted that as the collision energy increases, the relative intensity of the *m*/*z* 169 ion *decreases* and that of the *m*/*z* 91 ion increases. This is consistent with the fact that the intermediate, ion−neutral complex **3**, collapses more readily when the collision energy is higher and thus the reaction via the complex becomes less competitive. However, it is worthwhile to mention that conversion of the benzyl cation, formed upon expulsion of formaldehyde from complex **3**, to the tropyl ion is unlikely to occur at the collision energy employed ($E_{cm} = 0.3$ eV, Figure 4) even though multiple collisions[40] may be involved. The conversion would result in the observed changes in intensity of the product ions (since the tropyl ion may not be active in forming the *m*/*z* 169 ion); however, this process has a significant energy barrier (67.8 kcal/mol) as revealed in a recent theoretical study.[41] Furthermore, Holmes et al.[42] have even demonstrated that "pure" benzyl cation can be generated from benzyl derivatives at low energies.

Dual Mode. In some cases, the fragmentation reaction undergoes an intermediate proton-bound complex, from which the moiety of the molecule that gets the proton may either be lost as the neutral species or detected as the fragment ion. Previously, we have shown[19] that C_α-protonation of benzamides and other α,β-unsaturated amides leads to cleavage of the C_α−C(O) bond to form a proton-bound dimeric complex of isocyanate and benzene (or olefin). From the complex, benzene (or olefin), which keeps the original ionizing proton, may leave as a neutral species or get the proton shared in the complex. The kinetic method[43] was applied in the identification of the complex, and we were inspired to further explore the applications of the kinetic method to the studies of reaction mechanism including fragmentation reactions initiated by protonation.

In the CID mass spectrum of the MH^+ ion of acetophenone (*m*/*z* 121), a dominant product ion at *m*/*z* 43 was found along with a minor one at *m*/*z* 79. Obviously, they are formed as a result of the breakage of the C_α−C(O) bond. As discussed earlier, aromatic carbonyl compounds[23,44] are preferentially protonated at the oxygen. To trigger the C_α−C(O) bond cleavage, an intraionic proton transfer to the C_α-position is required although the proton transfer and bond cleavage may take place in either a stepwise or a concerted manner. As shown in Scheme 3, the incipient acetyl cation and the neutral benzene are associated as ion−neutral complex **4**, which subsequently

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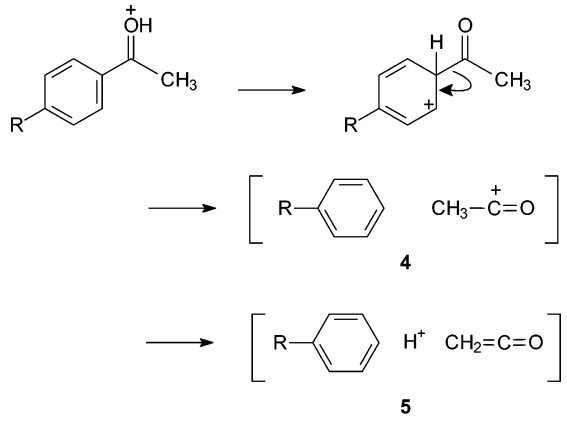
SCHEME 3



isomerizes to afford species **5**, a complex of ketene and benzene bound together by a proton. When R = H, the reaction channel giving CH_3CO^+ (*m*/*z* 43) is almost 19 kcal/mol more favorable than the other leading to *m*/*z* 79 as suggested by the thermochemical data in Table 1. This reaction is similar to the fragmentation of benzamides[19] and the kinetic method should be applicable to probe the intermediacy of the proton-bound complex in this reaction and, therefore, confirm the "dual mode" in relation to the fate of the external proton and its capturer.

The kinetic method[43] is a convenient approach in determining the PA (and other thermochemical properties) of organic molecules. Briefly, to evaluate the PA of molecule B_1, a series of reference molecules (B_2) is used to generate proton-bound dimers $[B_1\cdots H^+\cdots B_2]$, which dissociate to give both B_1H^+ and B_2H^+ competitively. The intensity of individual protonated monomers is so dependent on their proton affinities that the $\ln[B_2H^+/B_1H^+]$ values yield a linear relationship with the differences in PA of B_1 and B_2. To apply the kinetic method here, acetophenones bearing various substituents were subjected to collision-induced fragmentation. The acetyl cation and protonated benzenes were observed in all cases, but their intensities varied dramatically depending on how the substituent changes the PA of benzenes. A kinetic method plot was obtained as presented in Figure 5. The product ion intensity correlates well with the proton affinity of the related neutral fragments; a higher PA of the (substituted) benzene results in a higher intensity of the molecular ion of that benzene. Clearly, the fragmentation of the *molecular ions*, $[RC_6H_4COCH_3]H^+$, takes place as if the dissociating ions were the proton-bound *complexes* $[RC_6H_5\cdots H^+\cdots CH_2CO]$. Each of the two partners in the intermediate complex can either leave as a neutral species or obtain the shared proton and form the final product ion.

This result offers strong experimental evidence that the dimeric complex of ketene and benzene is the intermediate involved in this reaction, which explicates the fact that protonation at the C_α position triggers the fragmentation even though the local PA at this position is *lower* than that at the carbonyl oxygen. The C_α−C(O) bond, which is strong in the neutral molecule, becomes a crevice after the C_α-atom is protonated. In addition, it shows, once again, that the kinetic method can be applied in identifying a proton-bound dimeric intermediate in the fragmentation of molecular ions.

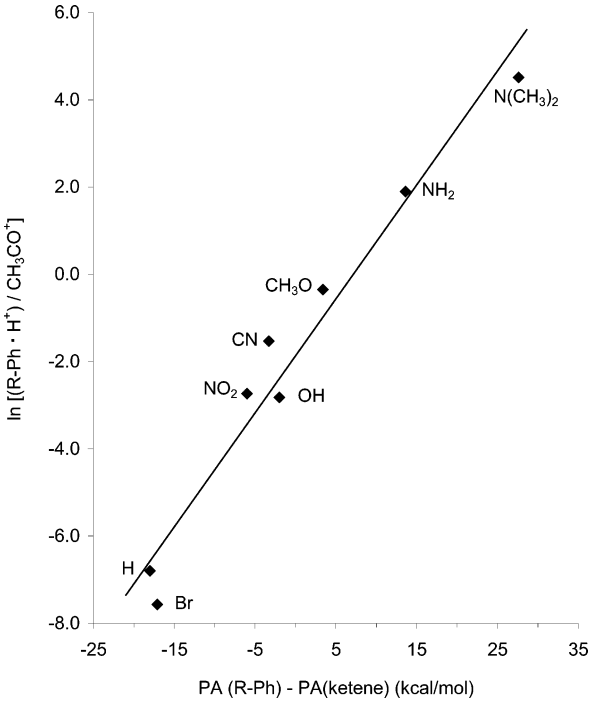


FIGURE 5. Kinetic method plot for the fragmentation of the MH^+ ions of para-monosubstituted acetophenones. Collision energy E_{cm} = 2.5 eV (nitrogen).

Conclusions

In the ESI-MS of benzophenones, acetophenones, and dibenzyl ether, we have observed deacylation and dealkylation, which are the retro-Friedel−Crafts reactions in the gas phase. These molecules are all preferentially protonated at the oxygen atom. For the two types of carbonyl compounds, no reactions take place when the proton is bound at the oxygen; for the ether molecule, only a minor α-cleavage is observed when the oxygen is protonated. In both cases, however, when the proton migrates to the aromatic carbon atom to which the carbonyl or the methylene group is attached, the major reaction (deacylation or dealkylation) is observed. It is demonstrated that a fragmentation reaction occurs when the proton reaches a *dissociative* protonation site, which is *not* necessarily the thermodynamically most favorable site for protonation.

Acknowledgment. The author thanks a reviewer for proposing alternative reaction pathways and comments on the overall reaction mechanisms.

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