Probing Electrostatic Effects: Formation and Characterization of Zwitterionic Ions and Their “Neutral” Counterparts in the Gas Phase

Katherine M. Broadus and Steven R. Kass*

Contribution from the Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455

Received May 15, 2000

Abstract: A dipolar ion and its nonzwitterionic counterpart were generated and characterized in the gas phase. A single charge site is found to be sufficient to lower the energy of a zwitterion well below its neutral counterpart. Charge separation in a dipolar anion enables hydrogen−deuterium exchange to take place with acids over an extraordinarily large range. These results provide a basis for studying electrostatic effects and understanding mass spectroscopic processes involving large biological molecules.

Introduction

Zwitterions, also known as dipolar ions, are molecules with oppositely charged sites. These species are used in a myriad of practical applications from synthesis and the construction of novel materials to buffers, enzyme inhibitors, and drugs.[1−6] They abound in nature as all 20 naturally occurring amino acids exist in dipolar form over a wide pH range. The resulting electric field plays a crucial role in determining the structure and reactivity of many proteins and enzymes. As a result, the consequences of electrostatics on molecular recognition and catalysis have been studied in solution, but aggregation, counterion, and solvent effects are difficult to factor out.[7−11] The physical environment in a solution also can be quite different from an enzyme’s active site. Computational and experimental gas-phase studies on zwitterionic species, consequently, are of considerable interest.

Calculations on glycine, the simplest amino acid, indicate that the dipolar form does not exist in the gas phase as a stable structure unless two or more water molecules are present.[12] Larger and more basic amino acids are capable of supporting a zwitterionic structure in some instances but the neutral (uncharged) form is more stable, even for arginine, despite an earlier claim to the contrary.[13−15] Gas phase experiments also indicate that amino acids exist in their neutral form, and consequently, only limited thermochemical results on zwitterions are available; basicities of betaine ($((CH_3)_2N^+CH_2CO_2^-)$) and *o*-trimethylaminobenzoate ($(o\text{-}(CH_3)_3N^+C_6H_4CO_2^-)$) have been measured by kinetic methods.[16,17] Many large peptide ions are believed to possess salt bridges and exist as dipolar ions,[18−22] but the evidence is largely circumstantial since the independent preparation of the corresponding nonzwitterionic forms has not been carried out, and the differences between the two are unknown. Herein, we report the first preparation and characterization of a gas-phase dipolar ion (**1**) and its corresponding nonzwitterionic (“neutral”) ion (**2**). Differences in the dissociation, reactivity, and thermochemistry of these species will be presented.

Experimental Section

Methods and Materials. 3,5-Dimethoxycarbonylpyridine and 3,5-dimethoxycarbonyl-1-methylpyridinium methyl sulfate were prepared according to literature procedures.[23] All reagents were used as received and solvents were dried via standard methods. NMR spectra were collected on Varian VXR-300 or VAC-300 spectrometers and are reported in ppm (δ). High-resolution mass spectra were obtained on a Finnigan 2001 FTMS by electrospray ionization. Solutions were prepared in methanol−water (35:65 v/v) with dilute ammonium hydroxide (2% v/v) and poly(ethylene glycol) (MW = ∼ 200 g/mol) as the reference.

3-Carboxy-5-methoxycarbonylpyridine (4).[24] 3,5-Dimethoxycarbonylpyridine (0.4 g, 2.1 mmol) was dissolved in 22 mL of a 0.1 N

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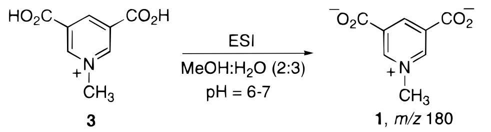
potassium hydroxide solution in methanol. After the mixture was stirred for 12 h at room temperature, any unreacted starting material was extracted with ether and the aqueous layer was concentrated by rotary evaporation. The product was recovered in 75% yield (0.28 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.12 (d, J = 2 Hz, 1H), 8.97 (d, J = 2 Hz, 1H), 8.57 (t, J = 2 Hz, 1H), 3.89 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.3, 166.2, 154.9, 150.0, 137.2, 136.2, 125.1, 52.8. HRMS ESI (M − H)$^-$ calcd for $C_8H_6NO_4$ 180.0302, obsd 180.0310.

1-Methylpyridinium-3,5-dicarboxylic Acid Hydrogen Chloride (3). 3,5-Dimethoxycarbonyl-1-methylpyridinium (0.18 g, 0.9 mmol) was dissolved in ~1 mL of concentrated hydrochloric acid, and the mixture was refluxed for 3 h. The precipitate that formed upon cooling was filtered and washed with cold water. The crude product was recrystallized from hot water to give 0.08 g (50%) of **3**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.69 (s, 2H), 9.08 (s, 1H), 4.49 (3, 3H), (300 MHz, NaOD/D_2O, pH 10) δ 9.02 (s, 2H), 8.96 (s, 1H), 4.34 (s, 3H); ^{13}C NMR (75 MHz, NaOD/D_2O, pH 10) δ 167.5, 146.8, 144.0, 136.7, 48.3. HRMS-ESI (M − H_2)$^-$ calcd for $C_8H_6NO_4$ 180.0302, obsd 180.0309.

3,5-Dimethoxycarbonyl-1-methylpyridinium-*d*$_3$ Methyl Sulfate. The deuterated compound was prepared according to a procedure for the protio material.[23] To a mixture of 3,5-dimethoxycarbonyl pyridine (0.22 g, 1.1 mmol) in benzene (5 mL) was added dropwise dimethyl sulfate-d_6 (0.7 mL, 3.8 mmol, Aldrich 99+% enriched). After refluxing for 3.5 h, the reaction mixture was cooled and acetone (~ 2 mL) was added. The precipitated product was collected by suction filtration to afford 0.25 g (91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.64 (d, J = 1.5 Hz, 2H), 9.32 (t, J = 1.5 Hz, 1H), 4.07 (s, 6H).

1-Methylpyridinium-3,5-dicarboxylic Acid-*d*$_3$ Hydrogen Chloride. 3,5-Dimethoxycarbonyl-1-methylpryidinium-d_3 methyl sulfate (0.24 g, 1.1 mmol) was hydrolyzed to its corresponding dicarboxylic acid following the procedure for the unlabeled material. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.69 (d, J = 1.5 Hz, 2H), 9.08 (t, J = 1.5 Hz, 1H). HRMS-ESI (M − H_2)$^-$ calcd for $C_8H_3D_3NO_4$ 183.04876, obsd 183.04879.

Gas-Phase Experiments. All experiments were carried out in a Finnigan Fourier transform mass spectrometer (FTMS) equipped with a 3.0 T superconducting magnet and interfaced with a custom-built Analytica electrospray ionization source (ESI) which contains an adjustable amplitude hexapole. Methylated pyridinium-3,5-dicarboxylate (**1**) and the conjugate base of 5-methoxycarbonyl nicotinic acid (**2**) were sprayed (3 μL/min) into the gas phase from 500 μM methanol−water (35:65 v/v) solutions of the corresponding diacid (**3**, pH 6−7) and monoacid (**4**, pH 8−9), respectively (eqs 1 and 2); dilute ammonium hydroxide (2% v/v) in methanol−water (~35:65) was used to adjust the pH. The following settings gave optimal signals: needle housing (cylinder) 2.6 kV, end plate 2.7 kV, capillary 5.9 kV (front) and −57 V (back), skimmer cone −13 V, acceleration lenses 335 V, deceleration lenses 21.4 V, and lens stack high voltage element 1.47 kV. Ions were accumulated in the hexapole for 0.80 s and transferred to the source cell in 30 μsec (TOF). Ion **5**-d_3 was generated from **1**-d_3 by exciting the *m/z* 183 ion to 1.4 eV (sustained off-resonance irradiation)[25] for 0.02 s concurrent with a 10^{-5} Torr pulse of argon.



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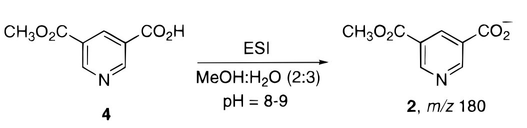

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Table 1. Dissociation Efficiencies for **1** and **2** upon Collision-Induced Dissociation (CID) and Sustained Off-Resonance Irradiation (SORI)

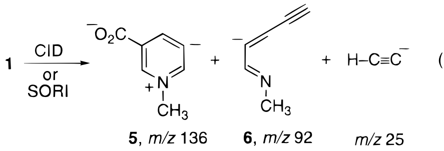
energy (eV)[a]	1			2		
	m/z 136	*m/z* 92	*m/z* 25	*m/z* 136	*m/z* 121	*m/z* 77
CID						
2.9	3.6	0	0	1.0	0	0
3.7	5.8	0	0	2.8	0	0
5.8	9.3	2.4	3.5	7.1	0.5	0.5
6.5	9.8	3.3	3.3	9.6	0.7	0.9
11.7	21.2	8.4	10.1	15.8	1.6	1.7
SORI						
3.9	1.5	0	0	0	0	0
9.8	15.3	4.8	5.2	11.2	1	1

[a] The energies have not been corrected for the ion's center of mass and the given values represent the product percentage relative to the amount of **1** (or **2**) after excitation. See eqs 3 and 4 for ion structures.

Ions of interest were isolated by ejecting undesired species (frequencies) using a SWIFT waveform or a broad band chirp excitation for low masses.[26] They were vibrationally cooled by collisions with argon which was pulsed into the cell up to pressures of 5 × 10^{-5} Torr. Reactions were carried out and monitored over time by introducing neutral reagents into the FTMS via pulsed or slow leak valves.

Results and Discussion

The zwitterionic ion **1** can be distinguished from **2** by the fragments observed when it is given energy in the presence of an inert gas (argon). This process is referred to as collision-induced dissociation (CID) or sustained off-resonance irradiation (SORI) if energy is not directly applied to the resonant frequency (mass-to-charge ratio) of interest.[25] Under both CID and SORI conditions, **1** primarily affords an ion corresponding to loss of carbon dioxide (**5**, *m/z* 136), but double decarboxylation (**6**, *m/z* 92) and fragmentation to acetylide ($HC{\equiv}C^-$, *m/z* 25) also are observed (eq 3).[27] In contrast, **2** gives ions at *m/z* 121 (M − CO_2 − CH_3)$^-$ and *m/z* 77 (M − CO_2 − CO_2CH_3)$^-$ in addition to a different species (see below) at *m/z* 136 (**7**, (M − CO_2)$^-$) (eq 4). The energy dependence also indicates that it is easier to break apart the dipolar ion than the "neutral" analogue under nearly single collision conditions (Ar ~ 1 × 10^{-7} Torr) using both on- and off-resonance irradiation (Table 1). This observation is consistent with dissociation studies of multiply charged ions and species that are thought to exist as salt bridges.[28−30]



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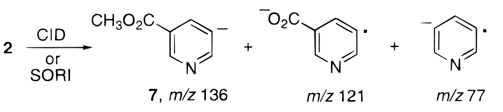

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[27] A ring-opened isomer of **5** ($CH_3N{=}CHC(CO_2^-){=}CHC{\equiv}CH$) also is produced but it can be minimized and never is greater than ~50%. The structural assignment for this ion is based upon a reasonable pathway for its formation (i.e. a ring-opening elimination of **5**), and the fact that it does not react with carbon dioxide in contrast to **5**−**7**, which add 1 equiv of CO_2. Ion **6** only adds 1 molecule of carbon dioxide and presumably arises from a ring-opening elimination.

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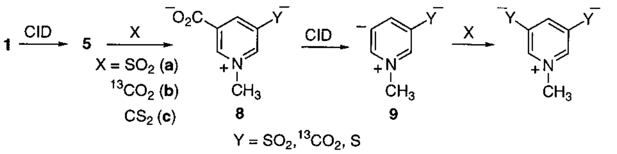
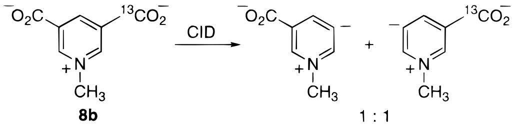
To verify the structure of **1**, the presence of two carboxylate groups must be demonstrated. This was accomplished by taking advantage of the reactivity of **5**. It is an aryl anion and ions of this type are known to react by nucleophilic addition with carbon dioxide and sulfur dioxide, while sulfur-atom abstraction takes place with carbon disulfide.[31,32] Accordingly, **5** was found to sequentially undergo these reactions twice as shown in eq 5.[33]



(5)

In the nonzwitterionic case, only one adduct or atom transfer product can be generated. Moreover, aryl ion **7** does not afford an $(M-CO_2)^-$ ion upon CID; fragments arising from the loss of carbon monoxide, methyl radical, and the methyl ester are detected, whereas **5** does lose carbon dioxide to afford **6**.

We have also shown that the two carboxylate groups in **1** are equivalent. A carbon-13 labeled species, **8b** (*m*/*z* 181), was synthesized as shown in eq 5. This ion was then isolated and fragmented at 1 × 10^{-7} Torr of argon to afford a 1:1 mixture of aryl ions arising from loss of labeled or unlabeled carbon dioxide (eq 6). In a similar fashion, **2**-^{13}C was prepared by addition of $^{13}CO_2$ to **7** and in this case exclusive loss of the label is observed upon dissociation.



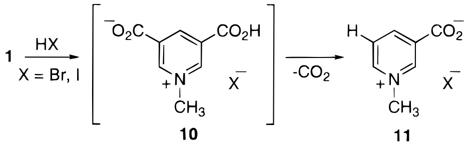
(6)

The electrostatic interactions in **1** influence its thermochemistry relative to that of **2**. We measured the proton affinity of **1** by the bracketing technique which involves monitoring the occurrence or nonoccurrence of proton transfer with reference acids. No reaction products are observed when **1** is allowed to react with ethoxyacetic acid or weaker acids (Table 2).[34] Upon reaction with hydrochloric acid, an adduct ion is formed. Dissociation of this product exclusively yields **1** which suggests the ion's proton affinity is less than that for Cl^-. As one progresses to stronger acids (HBr and HI) the adduct ion is no longer observed, but interestingly, an $(adduct-CO_2)^-$ ion (**11**) is detected (eq 7).[35] This product is also observed with HCl, but it is small (∼15%) in comparison to the clustered product. Presumably, **11** arises when the electrostatic interactions in the zwitterion−ion complex **10** are sufficiently large to induce fragmentation; collision-induced dissociation of **11** only gives bromide or iodide ion. Upon reaction with trifluoromethanesulfonic acid proton transfer is observed which suggests that the proton affinity of **1** is between that of this acid and HI (i.e., PA(**1**) = 310 ± 5 kcal mol^{-1}). This value is in excellent accord with a computed value of 311.0 kcal mol^{-1} (B3LYP/6-31+G(d)).[36] In contrast, **2** does not react with any of the acids studied to give an $(adduct-CO_2)^-$ ion under the same conditions employed for **1**. Its proton affinity also is between that of hydrochloric and hydrobromic acid (i.e., PA(**2**) = 328 ± 5 kcal mol^{-1}), which agrees well with the computed B3LYP value of 327.4 kcal mol^{-1}.

Table 2. Bracketing Results for the Proton Affinities of **1** and **2**

acid	ΔH°_{acid} (kcal mol^{-1})[a]	**1**	**2**
CH_3CO_2H	348.6 ± 2.9	no rxn	
$CH_3CH_2OCH_2CO_2H$	342.2 ± 2.1	no rxn	
HCl	333.4 ± 0.1	adduct (85%)[b] $(adduct-CO_2)^-$ (15%)	no rxn[c]
HBr	323.4 ± 2.1	$(adduct-CO_2)^-$	proton transfer
HI	314.4 ± 0.1	$(adduct-CO_2)^-$	
CF_3SO_3H	305.4 ± 2.2	proton transfer	

[a] Acidity values come from ref 34 except for HI which comes from Berkowitz et al.: Berkowitz, J.; Ellison, G. B.; Gutman, D. *J. Phys. Chem.* **1994**, *98*, 2744−2765. [b] CID of the cluster affords **1** as the sole product. [c] A cluster can be generated at high pressures, but not under the same conditions employed for **1**.



(7)

The smaller proton affinity of **1** is consistent with the fact that the remote positive charge stabilizes both carboxylates more than their mutual repulsion destabilizes them. Using a point charge model, where the nitrogen atom and the midpoint between the carboxylate oxygens are taken as the charge centers, leads to a significantly shorter distance for the attractive interactions than for the repulsive one (i.e., 4.28 vs 6.18 Å). Coulomb's law thus indicates that the proton affinity of **1** should be lower than that of **2** by 24 kcal mol^{-1} if a dielectric constant of 1 is used. This crude model overestimates the stability of **1** relative to the experimental (18 ± 7 kcal mol^{-1}) and computed (16.4 kcal mol^{-1}) differences, but is qualitatively useful. Isomerization of **1** to **2** is computed to be exothermic by 9.4 kcal mol^{-1} (B3LYP), but no evidence for methyl group migration was found in our work. It also is worth noting that the calculated energy difference between **1** and **2** is considerably smaller than that for **1H** and **2H** (25.8 kcal/mol) because of the favorable additional electrostatic interaction, but that the zwitterionic species are less stable than their nonzwitterionic forms in both of these cases.

The above experiments demonstrate that a zwitterionic ion can be generated in the gas phase by electrospray ionization and distinguished from its "neutral" analogue. As alluded to in the previous section, the decarboxylated fragments of **1** and **2** (**5** and **7**, respectively) can be differentiated as well. The most striking reactivity difference between the two aryl ions is the former's ability to undergo switching or ligand exchange

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(31) DePuy, C. H. *Org. Mass Spectrom.* **1985**, *20*, 556−559.

(32) Bierbaum, V. M.; DePuy, C. H.; Shaprio, R. H. *J. Am. Chem. Soc.* **1977**, *99*, 5800−5801.

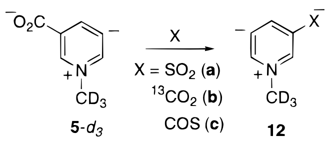
(33) Collision-induced dissociation of **8** gives a mixture of ions, and the desired product, **9**, was isolated and allowed to further react.

(34) All acidites, unless otherwise noted, come from the following: Bartmess, J. E. *Negative Ion Energetics Data in Secondary Negative Ion Energetic Data*, February 2000, National Institute of Standards and Technology: Gaithersburg, MD 20899 (http://webbook.nist.gov).

(35) We presume that carbon dioxide is lost from the carboxylate and then the acid proton migrates via a bimolecular process to afford the observed ion. Such behavior is consistent with ^{13}C-labeling studies which show CO_2 is lost from the charged site in $Ar(CO_2R)CO_2^-$ species. Reed, D. R.; Hare, M. C.; Kass, S. R. Submitted for publication.

(36) These calculations, which in all cases include a zero-point energy correction using unscaled vibrational frequencies, will simply be referred to as B3LYP hereafter. All of the computed acidities also include a temperature correction to 298 K so as to provide a direct comparison with experiment.

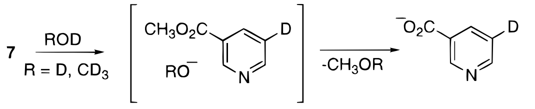
reactions with sulfur dioxide, carbonyl sulfide, and ^{13}C-labeled carbon dioxide (eq 8). These reactions occur at low pressures ($\sim 3 \times 10^{-8}$ to 2×10^{-7} Torr) of the probe reagents and without kinetically exciting the starting ion. Moreover, these processes are relatively facile as the intermediate is not necessarily observed. This behavior strongly indicates the presence of a second charge (reactive) site in the ion as the same reaction has been noted with sulfur dioxide and the benzoate distonic radical anions ($C_6H_4CO_2^{\bullet -}$), but not with benzoate anion.[37,38] Electrophile switching with the conjugate base of a Lewis acid–base complex, $^{-}CH_2S(CH_3)BH_3$, also has been reported.[39]



(8)

In the reaction of **5-*d*₃** with sulfur dioxide, oxygen-atom transfer ($(M + O)^-$, *m*/*z* 155) and an adduct ($(M + SO_2)^-$, *m*/*z* 203) also are detected. The exchange product (**12a**, *m*/*z* 159) behaves similarly with SO_2 to afford secondary product ions corresponding to oxygen-atom transfer (*m*/*z* 175) and adduct formation (*m*/*z* 223). The "neutral" equivalent (**7**) gives an oxygen-atom transfer product and an adduct, but does not switch CO_2 for SO_2. With high pressures of carbonyl sulfide ($\sim 10^{-6}$ Torr), **5-*d*₃** and **12c** afford adducts at *m*/*z* 199 and 215, respectively, while **7** yields a unique sulfur-atom transfer product (*m*/*z* 168, 40%) as well as an adduct (*m*/*z* 196, 60%). Last, **5** and **7** show divergent chemistry with carbon disulfide. Ions corresponding to abstraction of a sulfur atom are observed in each case; however, the $(\mathbf{5} + S)^-$ product fragments to lose carbon dioxide and undergo a second atom abstraction as shown in eq 5, whereas this is not the case for **7**.

Reactions of **5** and **7** with acids further illustrate that the two ions are unique and do not interconvert. The "neutral" ion reacts with deuterium oxide and methanol-*d*₄ to give the conjugate base of nicotinic acid and a small amount of methoxide-*d*₃ in the latter case. The former product forms by initial deuteron transfer followed by ester hydrolysis with the newly generated DO^- or CD_3O^- (eq 9). In addition, with D_2O one hydrogen can be exchanged for a deuterium to a minor (~14%) extent before the base attacks the ester group. Zwitterionic ion **5** undergoes acid-catalyzed isomerization with these reagents, and rearranges to **13** and/or a second species depending on the acid that is employed. We presume that **14** is the latter ion since it is the most stable aryl anion isomer (see below, Table 4). Upon reaction with deuterium oxide, **5** undergoes one hydrogen–deuterium exchange and a small amount of a second deuterium incorporation is observed at long (30 s at 4×10^{-8} Torr) reaction times (eq 10). To better probe this process, **5-*d*₃** was prepared and reacted with H_2O. Only a small amount of the label is washed out to give a *d*₂ ion (i.e., **13**), suggesting that **14** is the major product. In contrast, **5** undergoes 4 hydrogen–deuterium exchanges with methanol-OD (Table 3) and incorporates 3 additional deuteriums with methanol-OD when the *d*₁ ion generated from **5** and deuterium oxide is used as the substrate. Alternatively, when **5-*d*₃** is reacted with the deuterated alcohol 1 deuterium can be incorporated to a small extent, and when protio reagent is used all of the label can be washed out. These results indicate that methanol reacts with **5** and **14** to primarily afford **13**. In accord with these observations, we have found that **5**, **13**, and **14** undergo characteristic reactions with carbon disulfide. Ion **5-*d*₃** reacts with the reagent largely via sulfur-atom transfer, **14** (as the *d*₄ ion produced by reacting **5-*d*₃** with D_2O) is inert, and **13** (as the *d*₂ ion produced by reacting **5-*d*₃** with MeOH) affords the conjugate base of nicotinic acid (eq 11). The last of these reactions also occurs with carbonyl sulfide and presumably proceeds via sulfur-atom transfer and subsequent expulsion of thioformaldehyde.



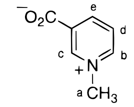
(9)

Table 3. Summary of Reactions Observed for the Zwitterionic Ion **5** with Reference Acids

acid	ΔH°_{acid} (kcal mol^{-1})[a]	H/D exchange[b]	proton transfer	S_N2[c]
D_2O	392.9 ± 0.1	1, 2nd small	no	no
CH_3OD	383.5 ± 0.7	3, 4th small	no	trace
$(CH_3)_3COD$	374.6 ± 2.1[d]	3, 4th small	no	trace
CF_3CH_2OD	361.8 ± 2.5[d]	4	no	minor
CH_3CH_2SH	355.2 ± 2.2	–[e]	no	yes
CH_3CO_2H	348.6 ± 2.9	–[f]	trace	yes
$CH_3CH_2OCH_2CO_2H$	342.2 ± 2.1	–	yes[g]	minor

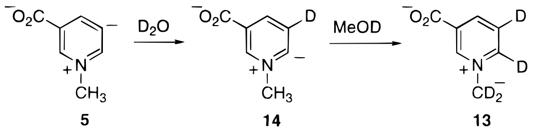
[a] Acidity values come from ref 34. [b] See text for details. [c] Refers to the acid's conjugate base attacking the methyl group of **5H** to give nicotinic acid. See text for details. [d] Values for protio acid used. [e] If the reaction is carried out with **5-*d*₃**, the deuteriums in the methyl group are exchanged for hydrogen. [f] With **5-*d*₃**, some washing out of the label is observed. [g] Proton transfer is the major reaction channel with this acid although some $(adduct\text{-}CO_2)^-$ is also generated.

Table 4. Relative Energies of $C_7H_6NO_2^-$ Isomers Computed at the B3LYP/6-31+G(d) Level of Theory (0 K) and Using Coulomb's Law ($\epsilon = 1$) with a Point Charge Model



compd[a]	E_{rel} B3LYP	E_{rel} Coulomb's law
a (**13**)	0.0	0.0
b (**14**)	1.2	8.1
c	16.9	48.0
d (**5**)	17.9	120.5
e	33.0	170.1
7	18.2	

[a] The letters represent the anionic site.



(10)

Zwitterion **5** incorporates three deuteriums with *tert*-butyl alcohol-OD, and at long times (5–10 s at 5×10^{-8} Torr) a fourth exchange is observed. With the stronger acid, trifluoroethanol-OD, this fourth exchange is more prominent because there is a closer correspondence between the acidities of the conjugate acids of **13** and **14** and the exchange reagent. The deuteriums in **5-*d*₃** can easily be washed out with ethanethiol and to some extent with acetic acid. Proton transfer is also observed in the latter case and occurs readily with ethoxyacetic acid; an $(adduct{-}CO_2)^-$ ion is detected as a minor product with the latter acid too. Likewise, **13-*d*₂** generated from **5-*d*₃** displays

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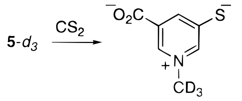
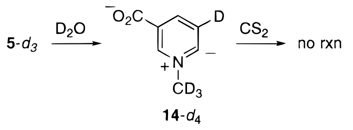

(11a)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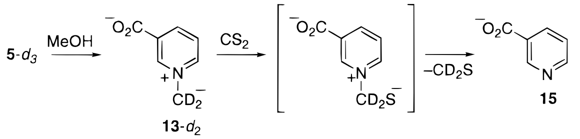
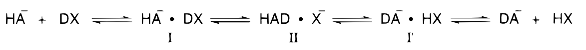

(11c)

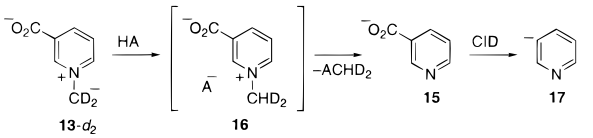
the same behavior with acetic and ethoxyacetic acids. These results lead to a bracketed proton affinity of 345 ± 3 kcal mol^{-1} for ylide **13** which is in good accord with the B3LYP predicted value of 343.8 kcal mol^{-1} and represents a remarkable acidifying effect of 68 kcal mol^{-1} relative to trimethylamine ($\Delta H^\circ_{\mathrm{acid}} =$ 412.8 kcal mol^{-1} (G2)).[40] The bulk of this stabilization is the result of the favorable electrostatic interaction between the formal positive charge on nitrogen and the negative charge on the dideuteriomethylene group.

Hydrogen−deuterium exchange between anions (HA^-) and deuterated acids (DX) has been extensively investigated.[41] It is well-known that the acidity of DX must be within 20 kcal mol^{-1} of that for H_2A for the process to occur. This requirement is a result of the mechanism (eq 12) that typically involves an endothermic proton transfer between DX and HA^-. This thermodynamically unfavorable step can take place because it is fueled by the ion−neutral complex energies of I and II which usually are on the order of 20 kcal mol^{-1}. When the initial proton transfer is less favorable than this amount there is insufficient energy in the system to drive this step and H/D exchange does not occur. In the case of **13** hydrogen−deuterium exchange is observed to slowly take place with deuterium oxide even though the initial proton transfer is endothermic by 47 kcal mol^{-1} and exchange is rapid with methanol-OD where the difference in acidity is still 37 kcal mol^{-1}! These unprecedented results at a carbon center are a consequence of the unusually large electrostatic interactions present in the ion−zwitterion complex (II, see **16** below).[42] This finding may account, at least in part, for the extraordinary deuterium incorporation that has been observed in large peptide ions upon reaction with deuterium oxide and methanol-OD where the proton affinity difference is on the order of 40 to 50 kcal mol^{-1}.[22,43]


$$\mathrm{HA^- + DX \rightleftharpoons \underset{I}{HA^-\cdot DX} \rightleftharpoons \underset{II}{HAD\cdot X^-} \rightleftharpoons \underset{I'}{DA^-\cdot HX} \rightleftharpoons DA^- + HX} \qquad (12)$$

Density functional theory calculations were carried out on **5**, **7**, **13**, **14**, and the other two dipolar aryl anion isomers (Table 4). Introduction of a single charge stabilizes the zwitterionic form relative to its "neutral" structure such that **5** and **7** are predicted to be essentially the same energetically. Dipolar anions **13** and **14** differ by only 1.2 kcal mol^{-1}, and both species are significantly (17−18 kcal mol^{-1}) more stable than the nonzwitterionic ion **7**. This result has obvious implications when considering the structures of large peptides and proteins in the gas phase. It is also worth noting that a simple point charge model using Coulomb's law is qualitatively useful in that it reproduces the relative DFT energies, and should be helpful in designing superacids and bases by virtue of electrostatic interactions.

An interesting reaction channel is observed when **5** is allowed to react with strong acids. Ethanethiol and acetic acid give rise to the conjugate base of nicotinic acid (**15**) via nucleophilic attack at the newly formed methyl group (eq 13). This pathway also slowly occurs with ethoxyacetic acid, trifluoroethanol, and methanol, which indicates that the proton affinity difference between **13** and A^- is a more important factor in determining if the reaction occurs than the strength of the nucleophile. The structure of the product was verified to be the conjugate base of nicotinic acid upon comparison with an authentic sample. In particular, under the same experimental conditions, both ions fragment by loss of carbon dioxide, and the resulting pyridyl ion (**17**) reacts with deuterium oxide to give ^-OD and two hydrogen−deuterium exchanges. This reactivity is in accord with that reported for the conjugate base of pyridine.[44] In addition to confirming the structure of **13**, this derivatization experiment supports the structure of our initial zwitterion **1**.


(13)

Conclusions

We have provided the first evidence that a zwitterionic ion and its "neutral" counterpart are unique species in the gas phase. The isomer pairs, **1** and **2** as well as **5** and **7**, exhibit thermochemical differences as well as divergent reactivity with probe reagents. Dipolar ion **5** undergoes acid-catalyzed isomerizations to remarkably stabilized ylide structures which also were explored. A single charge site is found to be capable of lowering the energy of a zwitterion relative to its neutral analogue such that the former species becomes more stable than the latter. Charge separation in a dipolar carbanion (A^-) enables hydrogen−deuterium exchange to occur with acids (DX) where the difference in acidity between HA and DX is extraordinarily large (i.e. 37−47 kcal mol^{-1}) and would normally not take place. This work provides a basis for studying electrostatic effects and additional results will be reported soon.

Acknowledgment. Support from the National Science Foundation, the donors of the Petroleum Research Foundation, as administered by the American Chemical Society, the Minnesota Supercomputer Institute, and the University of Minnesota−IBM Shared Research Project are gratefully acknowledged.

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[41] Squires, R. R.; Bierbaum, V. M.; Grabowski, J. J.; DePuy, C. H. *J. Am. Chem. Soc.* **1983**, *105*, 5185−5192.

[42] Binding energies of zwitterionic ions with neutral molecules should be similar to those for "normal" ions (i.e., ∼15−25 kcal/mol). In accord with this expectation, preliminary computations at 0 K at the B3LYP/6-31+G(d) (+ZPE) level indicate that the cluster energy of **5**·H_2O is 15.9 kcal/mol.

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