The Site of Ionization of Hydroxamic Acids Probed by Heteronuclear NMR Relaxation Rate and NOE Measurements. An Experimental and Theoretical Study†

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Abstract: The site of ionization (protonation and deprotonation) of hydroxamic acids (RCONHOH) has been investigated by heteronuclear (^{14}N, ^{15}N, ^{17}O) NMR relaxation and NOE experiments (R = Me, Ph) and *ab initio* theoretical methods (R = H, Me, Ph). Theoretical calculations indicate that nitrogen deprotonation is favored in all cases. Electric field gradient calculations have been used to estimate the change in nuclear quadrupolar coupling constants at O and N upon ionization and compared to experimental line width changes. NMR relaxation rate and NOE measurements in aqueous solution indicate that acetohydroxamic acid (R = Me) in water is predominantly an oxygen acid, whereas benzohydroxamic acid (R = Ph) is predominantly a nitrogen acid in methanol. Acetohydroxamic acid (m^* = 0.25, pK_{BH+} = −1.15) is protonated at the carbonyl oxygen.

Introduction

N-hydroxy, alkoxy, or aryloxy amides with the general formula RCON(R′)OR″ (R, R′, R″ = alkyl or aryl) are readily available by acylation of the corresponding hydroxylamine; the derivatives of hydroxylamine itself (RCONHOH) are generally called hydroxamic acids. Because of the ease of formation of metal complexes, these compounds are important in analytical chemistry;[1] some also have important biological properties.[2,3] Their chemistry has been reviewed by Bauer and Exner.[3]

As their name implies, hydroxamic acids behave as weak acids (pK_a's are in the range 8–9; in fact, their acidity is stronger than corresponding amides by some 6 pK units),[3] and, like most carbonyl compounds,[4] they are also weak bases.[3,5]

Their deprotonation equilibria have been extensively investigated both in solution[6-11] and in the gas phase.[12] However, a major point of concern comes from the fact that unsubstituted hydroxamic acids possess two sites of deprotonation, *i.e.* nitrogen and oxygen. The question concerning the actual site of ionization has been long debated. Thus, it has been proposed that the site of deprotonation is the nitrogen atom in MeOH,[6,9] DMSO,[10] and the gas phase,[12] though the possibility of *O*-deprotonation in hydroxylic solvents was not ruled out;[10] in fact, some results in water were in favor of *O*-deprotonation.[8] However, contrasting evidence has accumulated over the years, and to date no definitive conclusion has been reached; a recent paper by Exner *et al.*,[11] along with new measurements, summarizes the present situation and critically reviews the methods used. Thus, *N*-ionization has been inferred by UV spectral changes as a function of pH,[6] substituent effects,[6,10] comparisons with model compounds (*N*- or *O*-substituted hydroxamic acids),[6] and NMR studies, based on ^{17}O chemical shift changes.[9] Correlations between ΔH and ΔS of ionization[8] and some early studies[11] led to the opposite conclusion (*O*-ionization). A gas-phase acidity study[12] also favors *N*-ionization, though this stands apart from the others because of the different state. The picture is complicated also because these studies differ with respect to the structure of the acids employed, the experimental conditions, and especially the solvent (owing in part to the low solubility in water of aryl derivatives, studies have been carried out in various media such as water/methanol,[6] water/methyl cellosolve,[6] water/2-propanol,[11] water,[8] methanol,[9] and dimethyl sulfoxide[10]). The possibility that the site of deprotonation might depend on the solvent was indeed suggested.[8,10] The situation was finally summarized by Exner,[11] who stated that increased acid strength (*e.g.* by nitro substitution at an aromatic ring) and low-polarity solvents favor *N*-acidity, and *vice versa*.

Similar problems are encountered when one considers the protonation equilibrium, which in principle can take place at the carbonyl oxygen, at the nitrogen atom, or at the hydroxylamino oxygen. Because of the similarity of activity coefficient behavior of hydroxamic acids and other carbonyl bases (especially amides),[4] it is generally accepted that the site of protonation is the carbonyl oxygen.[5,13] On the other hand, it was claimed that a shift of the protonation site from N to C*O* takes place with increasing acidity, as was postulated for amides,[14a] and some recent IR studies[14b,c]

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are in favor of *N*-protonation. Gas-phase basicity studies did not yield conclusive information.[15]

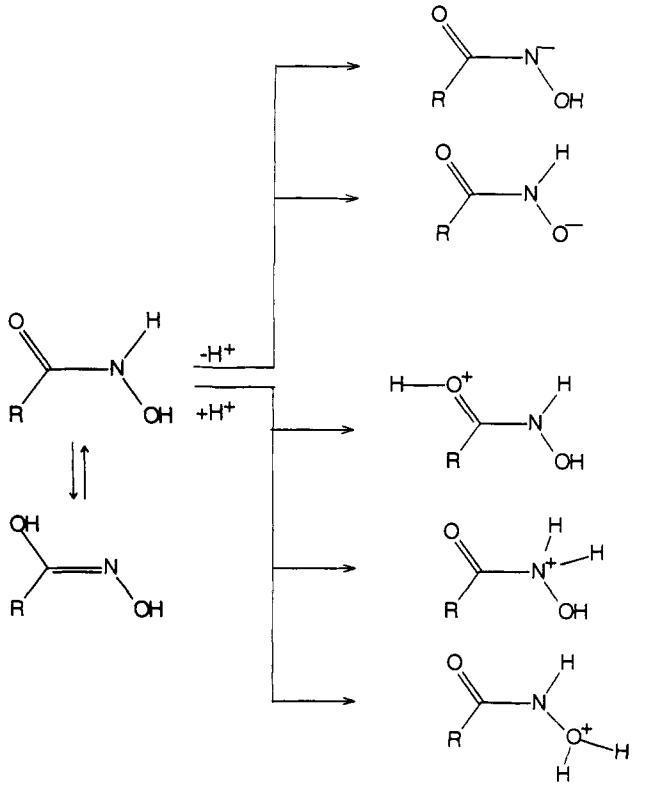
The appropriateness of some of the approaches to the problem of the ionization site has already been scrutinized;[11] nevertheless, we wish to remark the following points. (a) UV spectral changes do not provide clear-cut structural information of this kind, unless assumptions are made; for example, much of the early evidence in favor of *N*-protonation of amides[16] came from an erroneous interpretation of UV spectral changes. (b) Comparisons with model compounds (*e.g.* the acidities of RCONHOH and RCONHOMe) assume that the substituent effect of the methyl group is altogether negligible (though Exner[11] was able to partly overcome this problem). (c) The $\Delta H/\Delta S$ correlation was criticized as being incorrect.[11] (d) Trends in chemical shift changes do not provide structural data without recourse, once again, to model compounds or assumptions. Thus, when the ionization of benzohydroxamic acids is monitored by the ^{17}O chemical shift of the carbonyl oxygen, changes take place for PhCONHOH, PhCONHOMe, and PhCON(Me)OH; the proposed conclusion was based only on the similarity of the magnitude of the change for PhCONHOH and PhCONHOMe.[9] More generally, it is well-known that chemical shift changes upon ionization occur for all nuclei reasonably close to the involved site, but the magnitude and even the sign of the variation is difficult to predict.

Hydroxamic acids and their ions have also been investigated theoretically; however, high-level quantum mechanical calculations have appeared only very recently in the literature.[17-20] In most cases, these have dealt with simple derivatives, focusing attention on the relative energies of the various conformations available to this flexible system and its ions; the mechanism of acid-catalyzed hydrolysis has also been investigated.[18] All these works have emphasized the importance of using a large basis set and electron correlation in order to make realistic energetic predictions,[20] while zero-point energies gave minor effects.[19] A long-standing problem is the relative stability of the imidic acid [RC(OH)=NOH] and amide form; the latter is the only one experimentally found,[3,11] whereas theoretical results have shown that the most stable conformers of both tautomers differ very little in energy.[17,19,20] Theoretical results are relevant also in connection with the possibility of *cis-trans* isomerism (which is known to take place in DMSO solution[21]). With regard to ionization processes, currently available results consistently indicate that *N*-deprotonation (in agreement with gas-phase results[12]) and (C)*O*-protonation are the most favored ones.[18-20]

The overall picture (without regard for *cis–trans* isomers etc.) of the various acid–base and tautomeric equilibria is given in Scheme 1.

A well-established technique for the quantitative study of acid–base equilibria involves monitoring some spectral change (*e.g.* UV and IR absorbance, NMR chemical shift) as a function of some measure of the acid or basic strength of the solution (pH, acidity functions, etc.); this technique, however, is not guaranteed to furnish information about the structure of the ion(s) being formed. Studies in superacids, where ions can often be generated in a stable form,[22] may overcome this problem, but require drastic conditions that may affect the response if the solvation energies

Scheme 1



of the various ions differ greatly. Likewise, the analysis of substituent effects (*i.e.* trends in p*K*'s following substitution) yields structural information only through comparisons with model compounds, which assumes an exact similarity of behavior between various families of compounds.[11]

It is therefore apparent that none of the techniques employed so far can answer the question of the ionization site unambiguously. Recently, we have devised and tested a new NMR method which allows one to selectively probe ionization at a specific site;[23] the results of its application to the present case, together with related theoretical calculations, are the subject of the present paper.

Method

The method we have developed is based upon the fact that the NMR spin–lattice relaxation rate ($1/T_1$) of a given nucleus is affected by the addition or removal of a proton.[23]

In the case of spin-1/2 nuclei (*e.g.* ^{13}C, ^{15}N, ^{31}P, ^{77}Se), in the extreme narrowing limit the dipole–dipole contribution to T_1 (caused by fluctuating magnetic moments, generally due to nearby protons) is expressed by eq 1:[24]

$$1/T_1^{DD} = (4/3)S(S+1)Nh^2\gamma_X^2\gamma_H^2\tau_c/r_{XH}^6 \qquad (1)$$

Equation 1 shows that, apart from nuclear constants (proton spin quantum number S and magnetogyric ratios γ_X and γ_H) and the molecular correlation time τ_c, the efficiency of dipolar relaxation depends on the number of protons (N) located at a distance r_{XH}. Because of the extremely steep dependence on distance, the nucleus X will be sensitive essentially only to directly bonded protons. The outcome is that protonation of a given nucleus will increase its dipolar relaxation rate; if this pathway dominates the overall relaxation, this will also be reflected in the measured T_1 value. In any event, the sole DD contribution can be determined by measuring the nuclear Overhauser effect (NOE) η, because

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$1/T_1^{DD} = (1/T_1)(\eta/\eta_{max})$, where $\eta_{max} = \gamma_H/2\gamma_X$. Therefore, changes in T_1 of such nuclei are straightforward to predict.

Nuclei with $I > 1/2$ (*e.g.* ^{14}N, ^{17}O, ^{33}S) possess a quadrupole moment; the coupling between this and the electric field gradient (efg) existing at the nucleus due to the electronic distribution causes a very efficient relaxation of these nuclei, which is normally dominated by this pathway (eq 2 in the extreme narrowing limit):[24,25]

$$1/T_1 = 1/T_2 = (3/40)(2I + 3)/[I^2(2I - 1)]\chi^2(1 + \epsilon^2/3)\tau_c \quad (2)$$

$$\chi = e^2Qq_{zz}/h; \quad \epsilon = |q_{xx} - q_{yy}|/q_{zz} \quad (3)$$

In eq 2, χ and ϵ represent the nuclear quadrupolar coupling constant (NQCC), which is proportional to the largest principal component (q_{zz}) of the efg tensor q, and the asymmetry parameter of the latter, respectively (eq 3). Because of faster relaxation, NMR lines are generally much broader than in the previous case, and $T_1 \approx 1/(\pi W_{1/2})$, where $W_{1/2}$ is the line width. This version of the method is expected to work only if the acid–base reaction causes a change in the efg, thereby causing line widths to change. It is well-known that protonation at nitrogen causes a decrease of the efg;[25] in most other cases there is little if any information available, though criteria have been given for some nuclear arrangements.[26] Therefore, in such cases even the sign of change (if there is one) is not known *a priori*. However, such information can be supplied by theoretical calculations of the efg; even though these apply to isolated molecules, a net structural modification is expected to involve a larger change than the interaction with the solvent, and therefore a reasonable estimate can be provided anyway; obviously, this assumption has to be tested independently. Useful inference can be drawn also from model compounds (see below).

In both cases, the relaxation rates are proportional to τ_c. This essentially depends on the hydrodynamic molecular volume V_m (possibly including solvent molecules in the solvation shell) and the solution viscosity η (eq 4).[24] Viscosity and temperature can

$$\tau_c = \eta V_m/kT \quad (4)$$

be kept constant among different measurements; on the other hand, the possibility that V_m varies substantially upon ionization, thereby leading to an independent change in T_1, has to be considered; this will be discussed later.

In conclusion, this method amounts to measuring the T_1 of all conceivable ionization sites at two extreme values of acid or base strength, where either the neutral or the ionized form is present, keeping the viscosity and temperature constant. For non-quadrupolar nuclei this must be complemented by a heteronuclear NOE determination. In a previous communication, we demonstrated the validity of this approach in the case of some monofunctional models and polyfunctional compounds of known behavior.[23]

In this study, T_1's were determined by conventional procedures (see Experimental Section) wherever practical; ^{17}O measurements at natural abundance (0.037%) yielded only line widths, whereby values of $T_1 \approx T_2^* = 1/(\pi W_{1/2})$ were estimated.

Results

General Features of NMR Spectra in Aqueous Solutions. Alkanehydroxamic acids have been reported to exist as *cis* and *trans* conformers in DMSO by means of ^1H, ^{13}C, and ^{15}N NMR.[21] In order to check these results in aqueous medium, we recorded the ^1H, ^{13}C, and ^{15}N spectra of acetohydroxamic acid and its methyl derivatives at pH 1 and 12 in water. Contrary to the results in DMSO, only one set of signals was found at either pH value. Possible reasons for this are that (a) the two forms undergo fast exchange, (b) they have negligibly different chemical shifts (less likely, because three different nuclei have been examined), or (c) a single form is present. This finding also rules out the occurrence of imidic acid forms. The ^{13}C signals, especially that of the carbonyl carbon, undergo small changes, very similar in magnitude and direction to those found by Iwamura *et al.*,[9] and can be taken as further evidence for the absence of the imidic acid. This problem was not further pursued, and all spectral changes with acidity have been interpreted as arising only from the acid–base equilibrium.

Deprotonation Equilibria: (a) *N*- and *O*-Methylacetohydroxamic Acids. As stated before, to our knowledge there are no available experimental data concerning line width changes at ^{14}N or ^{17}O upon deprotonation even for simple species. Therefore, along with theoretical calculations (see below), we have firstly applied the method seen above to the two monofunctional derivatives, $CH_3C(O)N(CH_3)OH$ (an oxygen acid) and $CH_3C(O)NHOCH_3$ (a nitrogen acid). We emphasize that this approach does not involve a comparison of trends in acid strengths (which was criticized by Exner[11]), but only of line width changes. Because quadrupolar relaxation is normally an intramolecular process, whose efficiency decreases with the inverse third power of the distance,[27] these changes are expected to be little dependent on substitution, as long as the substituents do not differ too much electronically (*e.g.* H and Me) and are not directly bonded to the nucleus under investigation. All these acids are ionized quantitatively in dilute aqueous basic solutions;[9] therefore, the spectra of the anions were obtained at pH > 12, and those of neutrals at pH = 1; the viscosity of these solutions does not differ appreciably.[28] Chemical shifts and line widths for ^{14}N and ^{17}O are collected in Table 1.

(b) Aceto- and Benzohydroxamic Acids. The ionization of these compounds was monitored by means of ^{14}N, ^{15}N, and ^{17}O NMR. In order to achieve a better accuracy, samples enriched in ^{15}N (99.5%) or ^{17}O (*ca.* 5%) were employed; with such samples, T_1's could be determined more accurately by conventional methods. However, because of the low solubility of PhCONHOH in water, MeOH had to be used as solvent. Even so, the width of its broader ^{14}N lines could not be accurately determined. All results (chemical shifts, ^{14}N and ^{17}O line widths, ^{15}N NOEs) are again collected in Table 1. As expected, ^{14}N and ^{15}N chemical shifts were within experimental error of each other.[25]

Protonation Equilibrium of Acetohydroxamic Acid. The protonation equilibrium of this compound was determined in aqueous sulfuric acid. The resulting values were processed according to the excess acidity method,[4,29] thus obtaining $m^* = -0.25$ and $pK_{BH+} = -1.15$. An analysis of the distribution curves of B and BH^+ showed that quantitative protonation is achieved only in very strong acids; therefore, the protonated form was generated in trifluoromethanesulfonic acid (CF_3SO_3H), which is stronger and less viscous (*ca.* 3 cP) than 98% H_2SO_4 (*ca.* 20 cP), which avoids an excessive line broadening due to the τ_c term in eq 2. The spectrum of the neutral form was obtained in 22% *tert*-butyl alcohol/water, which has a similar viscosity.[30] ^{14}N chemical shifts and relaxation data are reported in Table 1.

Theoretical Calculations. The energies of formo- and acetohydroxamic acids and their ions were calculated at the MP2(FC)/6-311++G** level of theory, following a geometry optimization at the HF/3–21G (neutrals), HF/6–311G** (cations),

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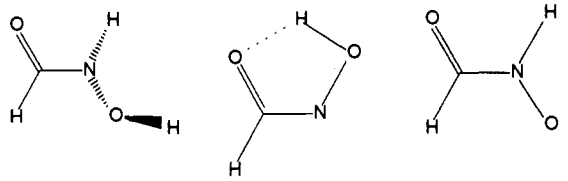
Table 1. ^{14}N, ^{15}N, and ^{17}O NMR Chemical Shifts and Relaxation Parameters for the Ionization of Hydroxamic Acids and their *N*- and *O*-Methyl Derivatives[a]

	^{14}N		^{15}N				^{17}O		
acid	$W_{1/2}$ (kHz)	T_1 (ms)	δ (ppm)	T_1 (s)	η	T_1^{DD} (s)	δ (ppm)	$W_{1/2}$ (kHz)	T_1 (ms)
MeCON(Me)OH[b,c]									
pH 1	1.7	0.14	−206				124	1.1	
pH 12	1.6	0.14	−200					not detectable	
MeCONHOMe[b,c]									
pH 1	1.5	0.14	−190				69	0.6	
pH 12	2.0		−148				81	0.6	
MeCONHOH									
pH 1	1.4	0.13	−214	12.1	−2.8	21.3	83	0.6	0.44
pH 12	1.7	0.12	−195	13.1	−2.9	22.2	150	1.1	0.24
22% *t*-BuOH[b]	2.1	0.04	−202						
CF_3SO_3H[b]	2.1	0.05	−185						
PhCONHOH									
MeOH/HCl	3.2		−211	4.1	−4.0	5.3	83	2.3	0.11
pH 12	2.7		−173	5.0	−2.4	10.1	146	2.0	0.13

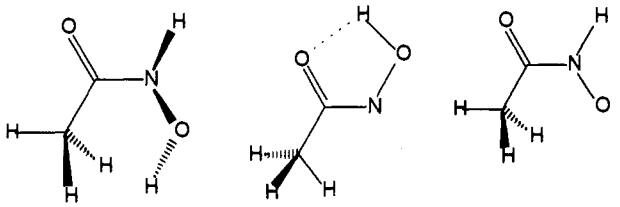
[a] ^{17}O measurements run in ^{17}O-depleted water. ^{15}N and ^{17}O measurements on enriched samples except where noted; only the hydroxylamino oxygen signal is listed. Note the different order of magnitude of the T_1 values for ^{17}O and ^{14}N (quadrupolar) and ^{15}N (nonquadrupolar). [b] Nitrogen chemical shifts measured in ^{14}N. [c] ^{17}O measurements at natural abundance.

Scheme 2

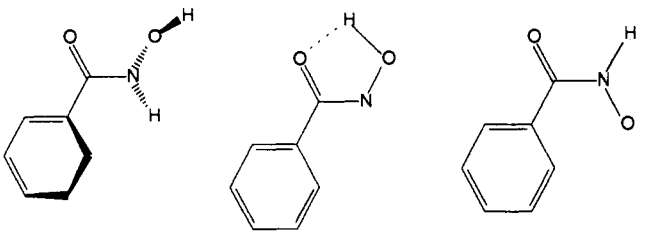
Formohydroxamic Acid and Anions



Acetohydroxamic Acid and Anions

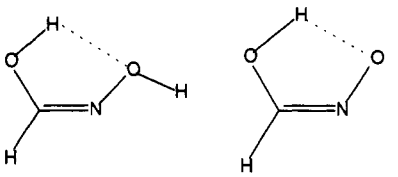


Benzohydroxamic Acid and Anions

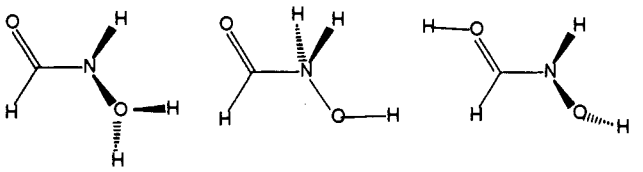


Scheme 3

Formohydroximic Acid and Anion



Formohydroxamic Acid (Protonated Forms)



or HF/6-311++G** level (anions).[31] The energies of benzo derivatives were calculated only at the HF/3-21G//3-21G level, with starting geometries similar to the most stable forms previously found for HCONHOH. The structures found to be most stable for each species investigated are depicted in Schemes 2 and 3, while the structures of less stable conformers, resulting from a complete conformational analysis, are collected as supplementary material.

Electric field gradients for formo- and acetohydroxamic acids and derivatives were calculated with a triple-ζ with polarization basis set (TZP),[32] whereas those of benzohydroxamic acid and its anions were calculated at the 3–21G level. This variant was shown to be sufficiently reliable by comparing the results for the formyl derivatives at both levels, which were found to be similar. Efg's are reported both conventionally, *i.e.* as principal component q_{zz} and asymmetry parameter ϵ, and as an effective NQCC, expressed as $\chi_{eff} = (eQq_{zz}/h)^2(1 + \epsilon^2/3)$, which is proportional to $1/T_1$ or $W_{1/2}$ (see eq 2). Energies at HF and MP2 level, calculated proton affinities ($E_{neutral} - E_{ion}$), and efg's for the species of Schemes 2 and 3 are in Table 2; atomic charges (from fits to electrostatic potential maps, resolution 2 points per atomic unit) at the level used to obtain the final energy are in Table 3, and relevant geometrical parameters are in Table 4. The energies and efg's of the remaining conformers are collected in the supplementary material.

The imidic acid tautomer of hydroxamic acids has never been detected in solution;[3] however, recent calculations[17,19,20] pointed out that it has a very similar energy compared to the amide form. The former may in turn deprotonate, giving rise to the anion RC(OH)=NO$^-$ (obviously, the structure RC(O$^-$)=NOH is just another canonical resonance form of the *N*-anion of the amide form). Therefore, analogous calculations were also carried out for formohydroximic acid and the corresponding anion.

Discussion

Despite the wide variety of methods so far employed, the structure of the anionic species arising from the ionization of hydroxamic acids has not been unambiguously determined. The problems related to such approaches have mostly been pointed out by Exner[11] and summarized in the Introduction.

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(32) Dupuis, M.; Maluendes, S. A. In *MOTECC-91: Modern Techniques in Computational Chemistry*; Clementi, E. Ed.; ESCOM: Leiden, 1991.

Table 2. Energies,[a] Proton Affinities (PA),[b] Electric Field Gradients,[c] and NQCC Changes for the Most Stable Conformers of Hydroxamic Acids and Ions

structure	E(HF)	E(MP2)	PA	ΔPA[d]	q_{zz}[e]	ϵ[f]	nucleus	χ_{eff}[g]	$\Delta\chi_{eff}$ %[h]
				Formohydroxamic Acid and Anions					
HCONHOH	−243.797 620	−244.515 183			1.788 319	0.190	C*O*	1.21	
					1.477 778	0.797	*N*H	0.41	
					−2.570 802	0.939	*O*H	3.19	
HCONOH⁻	−243.228 202	−243.956 902	350.321	(0.00)	1.223 396	0.939	C*O*	0.72	−40
					−1.369 013	0.372	N⁻	0.30	−27
					−2.152 271	0.980	*O*H	2.28	−28
HCONHO⁻	−243.213 829	−243.936 769	362.954	12.63	1.366 420	0.653	C*O*	0.80	−34
					0.790 421	0.328	*N*H	0.10	−76
					−3.485 131	0.071	O⁻	4.54	42
HC(=NOH)OH	−243.790 414	−244.513 017			−1.720 782	0.667	C*O*H	1.27	
					−1.265 612	0.850	*N*	0.31	
					2.333 068	0.951	N*O*H	2.64	
HC(=NO)OH⁻	−243.195 302	−243.929 570	366.11		−1.497 427	0.956	C*O*H	1.09	
					1.268 377	0.282	N	0.25	
					−2.754 497	0.233	N*O*⁻	2.88	
				Acetohydroxamic Acid and Anions					
MeCONHOH	−282.852 730	−283.720 728			1.783 976	0.127	C*O*	1.19	
					1.515 613	0.788	*N*H	0.43	
					−2.543 380	0.967	*O*H	3.17	
MeCONOH⁻	−282.280 635	−283.160 361	351.630	(0.00)	1.236 981	0.974	C*O*	0.75	−37
					−1.357 257	0.350	N⁻	0.30	−30
					−2.166 989	0.957	*O*H	2.29	−28
MeCONHO⁻	−282.266 439	−283.141 315	363.582	11.95	1.404 114	0.678	C*O*	0.85	−29
					0.859 947	0.294	*N*H	0.12	−72
					−3.432 362	0.044	O⁻	4.40	39
				Benzohydroxamic Acid and Anions					
PhCONHOH	−470.638 050				1.720 331	0.078	CO	1.11	
					1.354 162	0.643	*N*H	0.32	
					2.539 410	0.963	*O*H	3.15	
PhCONOH⁻	−470.056 617		364.85	(0.00)	−1.354 833	0.592	C*O*	0.76	−31
					−1.182 443	0.282	N⁻	0.22	−31
					2.278 881	0.899	*O*H	2.46	−22
PhCONHO⁻	−470.043 632		373.00	8.15	1.334 131	0.887	C*O*	0.84	−24
					0.776 985	0.020	*N*H	0.09	−72
					−3.894 025	0.094	O⁻	5.67	80
				Formohydroxamic Acid (Protonated Forms)					
HC(=OH)NHOH⁺	−244.132 534	−244.834 079	200.11	(0.00)	−1.650 333	0.287	C(*O*H)⁺	1.04	−14
					0.887 450	0.933	*N*	0.16	−61
					−2.867 550	0.803	*O*H	3.73	17
$HCONH_2OH^+$	−244.101 497	−244.810 411	185.26	14.85	1.869 804	0.954	C*O*	1.70	40
					−0.718 541	0.174	N	0.08	−80
					−2.910 237	0.839	*O*H	3.90	22
$HCONHOH_2^+$	−244.065 751	−244.771 550	160.87	39.24	1.875 196	0.707	C*O*	1.53	26
					−2.081 234	0.804	*N*	0.81	98
					2.301 873	0.269	*O*H	2.02	−37

[a] Energies (in au) at the 6-311++G** level for formo- and acetohydroxamic acids and anions and at the 3-21G level for benzohydroxamic acid and anions. MP2 energies in the frozen-core approximation. [b] Calculated proton affinity (energy difference in kcal mol^{-1} between ion and corresponding neutral). [c] At the HF/TZP level for formo- and acetohydroxamic acids and anions; at the HF/3-21G level for benzohydroxamic acid and anions. [d] Proton affinity relative to most stable ionic form. [e] Principal component of electric field gradient tensor in au (1 au = 0.97174×10^{22} V m^{-2}). [f] Asymmetry parameter (see eqs 2, 3). [g] Effective NQCC calculated as $(eQq_{zz}/h)^2(1 + \epsilon^2/3)$, in units of 10^{14} s^{-2}. [h] Percent change, relative to neutral.

Table 3. Atomic Charges for the Most Stable Conformers of Hydroxamic Acids and Ions[a]

structure	R	*C*O	C*O*	N	N*H*	N*O*	O*H*
HCONHOH	0.003	0.813	−0.648	−0.565	0.418	−0.431	0.409
HCONOH⁻	−0.165	0.893	−0.898	−0.714		0.503	0.387
HCONHO⁻	0.020	0.543	−0.835	−0.116	0.236	−0.848	
MeCONHOH	−0.044	0.959	−0.693	−0.615	0.409	−0.429	0.413
MeCONOH⁻	−0.262	1.010	−0.929	−0.675		−0.532	0.379
MeCONHO⁻	−0.041	0.682	−0.872	−0.183	0.227	−0.811	
PhCONHOH	−0.181	0.991	−0.609	−0.629	0.440	−0.475	0.463
PhCONOH⁻	−0.321	0.871	−0.821	−0.563		−0.573	0.408
PhCONHO⁻	−0.132	0.631	−0.759	−0.433	0.311	−0.618	

[a] Calculated from fits to electrostatic potential maps, using a grid of 2 points per atomic unit.

The method we have developed offers significant advantages. For nonquadrupolar nuclei, the change in T_1^{DD} is readily predictable in sign, and if the correlation time can be estimated with some accuracy,[24] even the magnitude can be calculated from eq 1. For quadrupolar nuclei in most species, the direction of change of the efg is not easy to predict; however, theoretical calculations are expected to provide estimates thereof. When this is not feasible, one can resort to model compounds to determine line width changes for known processes; we emphasize that in this context the use of model compounds only assumes a qualitative similarity (*i.e.* the sign rather than the magnitude) of the changes in a property which depends on the electronic symmetry at close range (owing to the r^{-3} dependence). However, the assumption that theoretical efg's can indeed be compared with experimental

Table 4. Geometrical Parameters of the Most Stable Conformers of Hydroxamic Acids and Ions[a]

species	distances						angles				dihedrals			
	CO	CH (CC)	CN	NH	NO	OH	OCN	OCH (C)	CNO	HNO	NOH	HNCH (C)	OCNO	CNOH	OCCH (C)
HCONHOH	1.206 827	1.078 678	1.374 990	1.001 653	1.436 927	0.968 904	124.09	124.70	113.12	112.30	104.90	157.0	−160.9	−116.0	
HCONOH⁻	1.249 351	1.096 373	1.291 102		1.409 601	0.947 674	128.96	119.72	110.54		105.11		p	p	
HCONHO⁻	1.231 189	1.092 703	1.301 767	0.998 309	1.356 320		126.94	121.47	125.31	117.64		p	p		
$CH_3CONHOH$	1.211 206	1.504 376	1.386 712	1.001 930	1.436 928	0.968 589	120.07	125.13	115.57	111.49	104.87	−159.1	157.6	117.2	2.2
CH_3CONOH^-	1.254 151	1.516 928	1.293 345		1.406 515	0.948 654	127.05	118.53	110.55		104.82		p	p	p
CH_3CONHO^-	1.235 765	1.513 363	1.307 513	0.997 278	1.355 424		123.97	121.08	125.94	117.79		p	p		−0.3
PhCONHOH	1.211 282	1.490 278	1.381 001	0.998 768	1.436 377	0.969 198	122.47	122.79	114.40	108.66	104.68	34.6	−15.1	83.7	17.6
PhCONOH⁻	1.275 758	1.505 185	1.298 360		1.474 780	0.977 609	127.30	118.49	108.66		102.41		p	p	p
PhCONHO⁻	1.255 862	1.503 823	1.312 388	1.001 268	1.466 738		123.06	118.25	130.83	114.96		p	p		p

species	distances					angles				dihedrals		
	(C)OH	CO	CH	CN	NO	(N)OH	HOC	HCO	OCN	CNO	NOH
HOC(H)=NOH	0.970 058	1.347 723	1.066 067	1.259 312	1.466 241	0.964 304	110.46	114.13	126.37	107.62	103.73
HOC(H)=NO⁻	0.960 398	1.358 154	1.074 535	1.257 404	1.334 562		100.05	115.80	121.56	113.49	

species	distances						angles					dihedrals			
	CO	CH	CN	NH	NO	(N)OH	OCN	OCH	CNO	HNO	NOH	COH⁺	HNCH	OCNO	CNOH	H⁺OCN
$HC(OH)NHOH^+$[b]	1.271 573	1.077 883	1.277 596	1.004 281	1.346 042	0.949 284	119.07	122.73	118.78	117.78	107.85	114.78	−174.1	−173.1	114.1	179.6
$HCONH_2OH^+$	1.144 472	1.084 154	1.548 740	1.014 187	1.363 185	0.950 575	117.13	133.14	107.62	112.05	109.71	–	±121.2	p	p	–
$HCONHOH_2^+$	1.158 506	1.086 633	1.442 136	1.005 361	1.426 932	0.960[c]	118.31	128.24	111.25	108.72	*d*	–	−152.3	155.9	*e*	–

[a] Distances in angstroms and angles in degrees. Entry "p" under dihedral means planarity to within ±0.05°. Both imidic acid structures are planar. [b] $(C)OH^+$ distance 0.949 572. [c] Exact distances are 0.959 626 and 0.958 368. [d] 118.952 and 112.443. [e] −89.1 and 133.49.

line widths must be tested (see below). In brief, this method probes the nuclear sites directly and selectively.

NMR Results. (a) Deprotonation Equilibria. We will firstly examine the two methylated derivatives (Table 1). Upon going from acid to basic solution, *N*-methylacetohydroxamic acid exhibits a small change in ^{14}N line width and a 6-ppm downfield shift. On the contrary, the ^{17}O signal undergoes a major broadening from 1.1 kHz to being so broad as to become undetectable (at natural abundance, this implies a line width > *ca.* 2 kHz). These data consistently indicate that *O*-deprotonation is accompanied by a large change in the ^{17}O spectrum, but not in the ^{14}N one. On the other hand, the ionization of *O*-methylacetohydroxamic acid involves a noticeable change in ^{14}N line width (from 1.5 to 2.0 kHz) and chemical shift (42-ppm downfield shift). The ^{17}O data are again consistent (small chemical shift difference, no line width change). Thus, model compounds indicate that *N*- or *O*-deprotonation should be detected by major changes in the ^{14}N and ^{17}O spectra, respectively. However, we note that the broad ^{14}N lines are difficult to measure accurately. We also note that in the case of *O*-methylacetohydroxamic acid a chemical shift difference at ^{17}O (12 ppm) is found without any line width change; thus the latter parameter shows itself to be a more selective probe of ionization, which cannot occur at oxygen in that molecule.

The results for acetohydroxamic acid again form a consistent set. The ^{14}N spectrum shows a moderate line broadening, but the more accurate T_1 values are equal; ^{15}N chemical shifts give a 19-ppm downfield shift. More importantly, ^{15}N NOEs remain constant, and so do the corresponding T_1^{DD} values. ^{17}O spectra reveal a marked downfield shift (67 ppm) accompanied by a line broadening (in this case it could be easily detected and even measured as T_1 because ^{17}O-enriched material was used). When these results are combined, it is evident that *O*-deprotonation is the dominant process.

Benzohydroxamic acid provides a different picture. ^{14}N results are very inaccurate because of large line widths, which now exceed 3 kHz (this is an effect of the correlation time, due to the higher molecular weight). However, the remaining data are sufficient; thus, in ^{15}N spectra we observe a 38-ppm downfield shift and especially a marked lengthening of T_1^{DD}, which demonstrates that the proton is lost from the nitrogen atom. This is further confirmed by ^{17}O, which features constant T_1's despite, once again, a noticeable chemical shift difference of 57 ppm.

As it was previously pointed out, a possible source of error may be due to the dynamics term in eqs 1 and 2, *i.e.* an independent change of the correlation time τ_c caused by ionization. This can happen for two main reasons: a different stability of the solvation shell, which changes V_m because hydrogen bonding to the solvent is stronger for an ion than for a neutral, or a change in τ_c itself due to a change in the molecular moment of inertia. The latter effect is expected to operate only on very small molecules, where protonation can drastically alter the molecular symmetry, *e.g.* NH_3 (C_{3v}) and NH_4^+ (T_d). The change in V_m depends on the number of solvent molecules that are bound to the solute for a long time on the rotational time scale. In any case, if we assume the solvation of the ion to be stronger than that of the neutral, τ_c will increase, and so will $1/T_1^{DD}$ and $W_{1/2}$. This implies that (a) for nonquadrupolar nuclei the efficiency of dipolar relaxation will increase, possibly leading to a decrease in T_1, which goes in the same direction expected for protonation; (b) the line widths of quadrupolar nuclei will increase. However, Perrin has found that the rotation of the ammonium ion in water is remarkably fast, despite the expected hydrogen bonding,[33] which points out that, even for a relatively strong hydrogen bond such as $N^+H\cdots O$, association with the solvent does not retard rotation appreciably. The above results are relevant with respect to this problem: in fact, upon deprotonation at nitrogen T_1^{DD} is lengthened, as

(33) Perrin, C. L.; Gipe, R. K. *J. Am. Chem. Soc.* **1986**, *108*, 1088.

expected from eq 1, and opposite to the possible effect of τ_c anticipated above. The results for ^{14}N and ^{17}O (line broadening) do follow the same trend as τ_c, but methylated derivatives have shown that when a given atom is not undergoing ionization, no major change in its line width takes place, despite the fact that V_m will change anyway. Thus, the overall picture is consistent with a minor (or at least not overwhelming) effect of the dynamics term relative to the structural term; however, a quantitative estimation of the relative importance is at present infeasible. It is possible that weaker interactions, such as hydrogen bonding itself, can be detected by the same technique in appropriate systems.

(b) Protonation of $CH_3CONHOH$. This was investigated by ^{14}N only. In this case, there is no difference between the T_1 values in neutral and acid solution. The chemical shift is not informative, because the solvents are now quite different. As *N*-protonation is expected to entail a large line narrowing (or T_1 lengthening),[25] we can conclude that acetohydroxamic acid is *not* protonated at nitrogen. The basicity of the hydroxylamino oxygen has been estimated[13a] to be lower than that of the carbonyl oxygen by some 7 pK units, which is confirmed by theoretical calculations (see below). Actually, protonation at CO might have been inferred by the magnitude of m^* and pK_{BH^+} (0.25 and −1.15, respectively), which are similar to those of acetamide (0.41 and −0.66, respectively);[34] the low m^* value is especially indicative of oxygen protonation.[4] The same conclusions can be drawn from the behavior of other hydroxamic acids;[5,13] however, NMR relaxation results provide independent evidence. The arguments put forward to propose *N*-protonation were essentially that there is an intramolecular hydrogen bond in the protonated form (from IR measurements),[14b,c] which is also compatible with *O*-protonation. Likewise, most evidence in favor of a shift of the protonation site of amides from N to O with increasing acidity came from either questionable assumptions on UV spectra[16] or the interpretation of the exchange rates of NH protons, which have been refuted by Perrin.[35]

Theoretical Calculations and Comparison with Results in Solution. In this work we have sought to extend the available calculations to the species experimentally studied, also because these present a fairly large structural change, the ultimate goal being to estimate the change in efg expected for the ionization at the various sites and to compare it with experimental line width changes. The need for large basis sets to accurately calculate efg's has been emphasized.[36] For these reasons, we have employed basis sets as large as practical, and we will comment only on MP2 energies where available.

With regard to the relative stability of the imidic acid and amide forms, we confirmed previous results, with energy differences that do not exceed 1.3 kcal mol^{-1}. However, the anions deriving from the imidic acid are higher in energy (by 20–30 kcal mol^{-1}) than those deriving from amide forms, so this question is irrelevant to the relative stability of the ionic forms, and will not be further pursued. (Actually, the most stable imidate structure was found to collapse to the corresponding amide form upon geometry optimization at MP2(full) level.[20])

The major factor affecting the relative stability of neutral acids is the configuration around the C–N bond, *E* forms being more stable than *Z* (by up to 10 kcal mol^{-1}) for R = H, Me; if R = Ph, the two have almost equal energy, presumably because steric repulsion between the OH and the *ortho* aromatic hydrogen destabilizes the former. In this case the phenyl ring is also tilted away from planarity (Table 4 and Schemes 2 and 3). The most stable structures also exhibit a nonplanar CONHO group,[17,19] whereas the conformation around the N–O bond has little effect on the energy.

With regard to deprotonated forms, we found that for all derivatives (a) the most stable form of the nitranion is planar with a *Z* configuration, strongly suggesting an intramolecular hydrogen bond; (b) the oxyanion is planar and *E* (see also refs 19 and 20). The stability order of the two anions is in favor of the nitranion by 8–13 kcal mol^{-1}, again in agreement with the results of refs 19–20. Therefore, theory predicts a higher acidity of the N–H proton, which agrees with experimental gas-phase results.[12] These results obviously refer to a situation very different from that in solution (and particularly in water or methanol) and therefore are not directly comparable to the latter. However, they do point out the different relative stability of oxyanions *vs* nitranions; the former are more strongly solvated than the latter ones[37] and therefore may be more stabilized in hydrogen-bonding solvents, depending on a sensitive balance between the "intrinsic" acidities (which are not very different) and solvation effects; the overall outcome is that hydroxamic acids may behave both as *N*- and *O*-acids, depending on their structure and, particularly, on the solvent. The possible influence of both factors was previously hinted at;[10,11] however, for the first time we have been able to directly detect both types of behavior in solution. Other estimates (coming from correlations of AM1 ΔH_f values with pK's in DMSO and water, and AM1 calculations on complexes with four water or MeOH molecules)[20] have led to contrasting conclusions. Thus, both aceto- and benzohydroxamic acid were estimated to be mainly *N*-acids in DMSO, with *O*-acidity more favored for the latter. Conversely, predominant *O*-acidity in water, due to preferential stabilization of the oxyanion, was inferred for both acids; this stabilization is least for benzohydroxamate, which points to preferential *N*-deprotonation. While the general outcome agrees with the expectations concerning the relative stability of nitranions and oxyanions, the influence of structural variations is less clearly defined.

A rationale for the different behavior experimentally found in water and methanol can be given as follows. Atomic charges (Table 3) indicate that in the nitranions the negative charge is largely delocalized, especially on the CON(H)O grouping, the R group accounting for *ca.* 10% of the total negative charge. On the contrary, in the oxyanions it is mostly localized on the two O atoms, with a minor contribution from R (when R = H, it is actually slightly positive). These results again confirm that the nitranion, having the most delocalized charge, is the most favored ion in the gas phase (or other poorly solvating media), whereas the oxyanion should be quite stabilized in hydrogen-bonding solvents. We also note that, in the series H, Me, Ph, the negative charge on the oxyanion tends to be better spread; for example, the charge on the two O atoms amounts to 93% of the total when R = H, and to 69% when R = Ph. Thus, the conditions favoring *O*-ionization in water are less marked for the latter compound, which is probably sufficient to alter this very delicate balance.

The site of protonation of HCONHOH is also clearly borne out by theoretical results, which favor the carbonyl-protonated form, in analogy to amides. The difference between this and the *N*-protonated isomer was calculated to be 16.9 and 18.4 kcal mol^{-1},[18,19] while our results, obtained at a higher level (and also with slightly different conformations), reduce this value to 14.8 kcal mol^{-1}. The form protonated at the hydroxylamino oxygen lies much higher in energy (39.2 kcal mol^{-1}) and need not be further considered. In this case there is no reversal of the protonation site in solution, which can be rationalized as follows. Solvation energies of protonated hydroxamic acids (RC(OH)$^+$NHOH) are not available, but protonated amides (RC(OH)$^+$NHR′) can be taken as models. Obviously, there is no experimental value for the corresponding *N*-protonated form (RC(O)NH$_2^+$R′), so the latter can be approximately modeled by the

(34) Bagno, A.; Lovato, G.; Scorrano, G. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1091.
(35) Perrin, C. L. *J. Am. Chem. Soc.* **1986**, *108*, 6807 and references cited therein.
(36) Cummings, P. L.; Bacskay, G. B.; Hush, N. S.; Ahlrichs, R. *J. Chem. Phys.* **1987**, *86*, 6908.
(37) Taft, R. W. *Prog. Phys. Org. Chem.* **1983**, *14*, 247.

corresponding ammonium ion (RNH_2^+R'). If we compare the values of the electrostatic contribution to the free energy of solvation[34] (in order to partly compensate for the different size), for R = R′ = CH_3 we find values of −53 and −60 kcal mol^{-1} for the protonated amide[34] and amine,[37] respectively. Thus, the 7-kcal difference in favor of the latter is not sufficient to overcome the greater intrinsic stability of the *O*-protonated form.

A point of interest was to ascertain whether electric field gradients calculated by means of *ab initio* methods are sufficiently accurate to predict the changes in the NMR line widths of the quadrupolar nuclei involved. The conformational analysis shows that the conformation dependence of the calculated efg is not very large, with maximum differences between 2 and 30%; however, for consistency we will discuss only the values for the most stable conformers (Table 2). The differences due to substitution (R in RC(O)NHOH) also do not cause major changes for R = H, CH_3, and Ph. This finding shows that even the simplest member of the family yields representative values and apparently reflects the r^3 dependence of this quantity. Therefore, major differences arise only from the ionization process. Hereafter, we will compare calculated efg's with line widths calculated from the more accurate T_1 values, where available ($W_{1/2} = 1/(\pi T_1)$).

Upon deprotonation, the efg at C*O* is predicted to decrease (with respect to the neutral acid) by 20–40% regardless of whether *O*- or *N*-ionization takes place. A comparison with experimental data is difficult, because the carbonyl oxygen was not labeled; however, from the spectra run at natural abundance in the methylated derivatives it seems that no major change occurs for the width of the carbonyl oxygen signal. With regard to the nitrogen atom, theory again predicts a change regardless of the actual ionization site, *i.e.* a decrease by roughly 30% for deprotonation at nitrogen itself and a larger one (>70%) for deprotonation at oxygen, thus, this parameter is not very informative. Efg's at the hydroxylamino oxygen are more interesting because a moderate decrease (20–30%) is predicted for deprotonation at nitrogen, and a large increase (40–90%) for deprotonation at itself, with an opposite trend for the two cases. These results contrast with the experiments on the methylated derivatives, where deprotonation at N or O induces a noticeable line width *increase* only at the ionizing nucleus. However, the predicted change at oxygen for deprotonation at itself agrees with experiment. For $CH_3CONHOH$ deprotonation, the N width remains almost constant (+8% with respect to neutral), and that of O increases by 83%; again, there is a large discrepancy concerning nitrogen, but *O*-deprotonation is borne out by the oxygen data. For PhCONHOH, the experimental N and O widths decrease slightly (−16 and −15%, respectively). The value for O is equal to the calculated one, while that for N at least goes in the same direction predicted for *N*-deprotonation; thus, in this case the agreement is much better, perhaps accidentally.

The reasons for this discrepancy may be solvation effects other than those due to molecular dynamics, *i.e.* intermolecular efg's due to solute–solvent interactions. These have been theoretically studied for some neutrals, where solvation by one water molecule induced a moderate decrease (3–10%) in the efg at the involved heteroatom;[38] these effects seem to affect mainly the nitrogen nucleus. However, charged species may behave differently.

With regard to protonation, and leaving aside the unlikely (N)*O*-protonated form, opposite changes are predicted for the CO efg upon protonation at itself (−14%) or nitrogen (+40%); the efg at the (N)O oxygen also does not change appreciably. Quite interestingly, the efg at nitrogen is predicted to decrease upon protonation at both sites, the decrease being larger for *N*-protonation, as in simple amines.[25] The change calculated for the *O*-protonated form is probably related to the involvement of the N atom in delocalizing the positive charge formally residing on oxygen, thereby engaging the lone pair (which is normally responsible for most of the efg at nitrogen).[25] These changes are not sufficiently selective and contrast with the experimental finding (^{14}N line width is constant, despite the fact that protonation does take place, which strongly suggests no involvement of nitrogen).

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Conclusions

The determination of the ionization site of polyfunctional bases and acids is a commonly encountered problem and one which has been investigated with a variety of methods; the deprotonation site of hydroxamic acids has provided a challenging example, due to its extreme sensitivity to structure and solvent. The analysis of NMR relaxation rates has proved to be selective and reliable in this type of problem; the popular method of comparing chemical shift changes in this case gives confusing results, whereas relaxation rates provide a complete and consistent picture. One drawback arises from the fact that the method does not lend itself easily to quantitative analysis. Therefore, the chemical conclusion that should be drawn from these results is that in water the *dominant* site of deprotonation is the oxygen for acetohydroxamic acid and, in methanol, the nitrogen for benzohydroxamic acid. While the two solvents are not exactly equivalent as to their solvating ability etc., they are the closest in practical terms, and it is noteworthy that opposite types of behavior were detected in so similar media. Obviously, this does not rule out the possibility that a larger change in solvent or substituent may reverse the balance; in this sense, there is no generally valid answer to the question sought. A straightforward extension of this study should in fact be to investigate this problem in different solvents, as long as viscosity permits having manageable line widths. The different site of ionization in the two cases can also be rationalized on the basis that *O*-ionization of $CH_3CONHOH$ gives rise to an oxyanion with a localized charge (which is well stabilized in water), whereas for PhCONHOH the aromatic ring can provide an extra stabilization of the nitranion, which apparently compensates for the loss of solvation. Calculated electric field gradients are not very successful in predicting experimental line widths; this may be due to several factors, notably solute–solvent interactions. However, more data are needed, especially for simpler molecules, in order to make an assessment. Such work is currently in progress.

Experimental Section

Materials. $CH_3CONHOH$, PhCONHOH, CH_3ONH_2·HCl (Aldrich), CH_3NHOH·HCl (Janssen), $^{15}NH_2OH$·HCl (99% ^{15}N), ^{17}O-depleted water (99.99% ^{16}O), and $H_2{}^{17}O$ (D_2O, 11% ^{17}O, 27% ^{18}O) (CIL) are commercial products. *N*- and *O*-methylacetohydroxamic acids were prepared by acetylation of the corresponding commercial hydroxylamine, according to literature methods.[39,40] ^{15}N-labeled aceto- and benzohydroxamic acids were prepared by acylation of $^{15}NH_2OH$·HCl with acetyl chloride or benzoyl chloride. The corresponding ^{17}O-labeled acids were prepared as above, using $NH_2{}^{17}OH$·HCl; the latter was prepared by reduction of nitrite, labeled by exchange with $H_2{}^{17}O$, with Me_2S/BH_3 in THF, and purified via the acetoxime.[41] For ^{17}O measurements, ^{17}O-depleted water was used as solvent. Acidic (HCl) and basic (NaOH) solutions were prepared by saturating it with gaseous HCl or by dissolving sodium metal, respectively. Trifluoromethanesulfonic acid was distilled before use.

NMR Measurements. Heteronuclear and relaxation measurements were run at 25 °C on a Bruker AM 400 instrument at 9.4 T (operating at 28.92, 40.56, and 54.24 MHz for ^{14}N, ^{15}N, and ^{17}O, respectively) equipped with a 5-mm broad-band probe; some ^{15}N measurements were also run on a Bruker AC 200 (4.7 T). Typical acquisition parameters for the above nuclei in their respective order were spectral window, 25, 3.3, and 25 kHz; $\pi/2$ pulse length, 29, 12.5, 15 μs; data points in acquisition, 512, 16K, 1K. Samples for ^{15}N measurements were degassed before use with freeze–pump–thaw cycles, cooling in an ice-water bath. Chemical shifts are referred to external CH_3NO_2 (^{14}N and ^{15}N) and water (^{17}O).

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Line widths were determined by Lorentzian fitting. ^{14}N and ^{17}O measurements (at natural abundance) were run on 1 M solutions of the substrate; ^{17}O and ^{15}N spectra on enriched compounds were obtained in 0.5 M solutions. In all cases, the substrate was dissolved in 0.1 M HCl or NaOH; in the latter case, the pH was adjusted to the desired value (>12 to ensure complete deprotonation; see, for example, the titration curves obtained by Iwamura *et al.*).[9] ^{15}N T_1 measurements were carried out with the saturation–recovery sequence, using 12–16 delay values between 0.01 and 90 s. NOEs were determined with nonselective proton saturation, with an irradiation time of 4–5 times the previously determined T_1. ^{14}N T_1 measurements were carried out with an inversion–recovery sequence incorporating acoustic ringing suppression,[42,43] using generally 12–16 delay values covering up to 5 times the T_1 value estimated from line widths ($T_2^* = 1/(\pi W_{1/2})$). ^{17}O T_1 measurements could only be carried out on the enriched compounds. These were done by inversion–recovery as before; acoustic ringing suppression was not needed, because the enrichment resulted in an apparent suppression of the carbonyl signal, which lies at *ca.* 270 ppm, thus allowing use of a smaller spectral window and a correspondingly longer preacquisition delay. The sharper (slowly relaxing) water signal was also efficiently suppressed in the inversion–recovery.[44] The alkylated derivatives were not enriched, and normal ^{17}O spectra were obtained with acoustic ringing suppression.[42] Use of ^{17}O-depleted water as solvent permitted longer accumulations (generally (6–7) × 10^5 transients) before memory filling, and ^{17}O-depleted water was also used for enriched compounds.

Benzohydroxamic acid is very sparingly soluble in aqueous solutions and exhibits substantially broader ^{14}N and ^{17}O signals; therefore, ^{14}N and ^{17}O T_1's were estimated from line widths determined in spectra obtained in MeOH with acoustic ringing suppression.

The protonation equilibrium of $CH_3CONHOH$ was studied at 25 °C in aqueous H_2SO_4; the chemical shift of the methyl ^1H signal (Bruker AC 200), relative to internal trimethylammonium sulfate, was monitored as a function of the acid concentration. The ^{14}N T_1 was determined with the above methods.

From the examination of ^1H and ^{15}N spectra, we found that hydrolysis of the acids occurred only upon standing for several days in acid or alkaline solution; using only freshly prepared samples prevents this problem.

Theoretical Calculations. For the conformers of formo- and aceto-hydroxamic acids, geometry optimizations were carried out at the HF/3–21G level of theory, while for the corresponding anions and cations this was done at HF/6-311++G** and 6–311G** levels, respectively, following a preliminary optimization at the 3–21G level. Geometry optimizations of the neutrals were carried out with standard initial geometries (*e.g.*, planar CONH group) and without any symmetry constraint; the starting geometries of the anionic forms were those of the corresponding neutral with the desired hydrogen removed. The protonated forms of HCONHOH were calculated starting from a structure similar to the most stable form of the neutral species. Final energies were calculated at the MP2/6–311++G** level in the frozen-core approximation, and electric field gradients at the HF/TZP level. For benzohydroxamic acid and anions, HF/3–21G//3–21G energies and efg's were calculated. Energies were calculated with the *Spartan* V. 2.1 program, and efg's with *HONDO8* from the MOTECC package,[32] both running on an IBM RS/6000 workstation.

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Supplementary Material Available: Calculated structures, energies, and efg's of all conformers (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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