Solvent effect on relative *N*- and *O*-acidity. Inversion of the deprotonation site of 2- and 4-[(2,4,6-trinitrophenyl)amino]benzoic acids

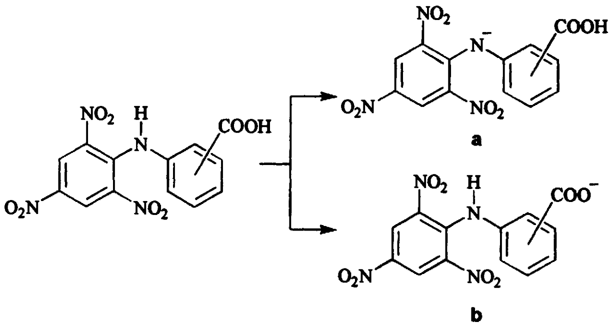
Alessandro Bagno,* Fabrizio Dorigo, Patrick McCrae† and Gianfranco Scorrano

Centro CNR Meccanismi Reazioni Organiche, Dipartimento di Chimica Organica, Università di Padova, via Marzolo 1, 35131 Padova, Italy

The deprotonation equilibrium of the title compounds (1 and 2, respectively) was experimentally investigated in DMSO–water (DMSO = dimethyl sulfoxide) mixtures by means of ^{15}N NMR relaxation, and theoretically by semiempirical (AM1) and *ab initio* (HF/3-21G) calculations, as isolated species and in dimethyl sulfoxide or water using continuum solvation models (AM1–SM2 and SCRF). Calculations predict the ionization to take place at the nitrogen atom for both acids in the gas phase. AM1–SM2 results again indicate the nitroanion to be more stable in water, whereas SCRF calculations predict the oxyanion to be slightly favoured over the nitroanion in both solvents. NMR results indicate that the preferred ionization site of 2 is the amine nitrogen in Me_2SO and the carboxylic oxygen in Me_2SO–water 60/40, whereas deprotonation of 1 leads to a nitroanion with an intramolecular $N^- \cdots H$–O hydrogen bond.

The site of protonation or deprotonation of species which possess more than one available site[1] depends on the relative stability and solvation of the ions that can be formed. For example, it is known that an aliphatic sulfide like Me_2S is quantitatively protonated in 96% sulfuric acid, whereas the corresponding ether Me_2O is not, despite the fact that their relative basic strength expressed by aqueous p*K* values is largely in favour of the latter (*ca.* −7 for sulfides and −2 for ethers).[2] This paradox is rationalized recalling that oxonium ions are more strongly solvated than sulfonium ions (the free energies of hydration of Me_2OH^+ and Me_2SH^+ are −84 and −55 kcal mol^{-1}, respectively; 1 cal = 4.184 J),[3,4] and therefore an oxonium ion will predominate only if an appropriate solvent is available to stabilize it. If this is not the case, the weakly solvated sulfonium ions will be preferentially formed instead, which is what happens in concentrated acids, where the water content is low.[2]

A few attempts have been made to detect an inversion of the relative acidity or basicity of different sites within the same molecule caused by solvent changes.[1] Of these, the one due to Terrier *et al.*[5] is perhaps the most intriguing. These authors investigated the ionization of 2-, 3- and 4-[(2,4,6-trinitrophenyl)amino]benzoic acids in DMSO–water (DMSO = dimethyl sulfoxide) mixtures. These acids have two sites available for deprotonation, *i.e.* the carboxylic proton and the amino proton, and can, therefore, give rise to a nitroanion (**a**) or an oxyanion (**b**) (Scheme 1). The tautomeric equilibrium was studied with a potentiometric technique, but a quantitative interpretation of the results was hampered by the fact that the apparent ionization constants cannot be simply related to the true equilibrium constants; the authors had to resort to several assumptions regarding the medium dependence of the p*K* of the various equilibria involved. Each ionization constant was assigned to a specific process by comparing UV spectral changes occurring upon increasing the medium basic strength with those of analogous monofunctional acids (polynitrodiphenylamines). All these data led the authors to conclude that in DMSO–water mixtures oxygen deprotonation occurs in the water-rich region, and nitrogen deprotonation in DMSO-rich mixtures; the inversion point was estimated to lie around 80–85% DMSO.[5]



Scheme 1

This finding can be rationalized recalling the large and opposite solvent effect on the ionization of the parent acids: benzoic acid becomes weaker on going from water (p*K* = 4.21) to DMSO (p*K* = 10.9),[6] whereas (2,4,6-trinitrophenyl)phenylamine becomes a stronger acid on going from water (p*K* = 10.55) to 95.5% DMSO (p*K* = 7.97).[7] However, parent species may not be adequate models of **1** and **2**, owing to their more extended π system, and the above experimental difficulties have left some problems still open. Hence, we were prompted to re-investigate the problem by a totally different approach. In fact, we have recently developed and tested an NMR method[8] aimed at determining the structure of the ion(s) formed in an ionization process, which is based on the changes in the NMR relaxation rate of the atoms which can conceivably act as acidic sites.

The aim of this study is therefore to ascertain whether a change in solvent composition (from pure DMSO to DMSO–water mixtures) causes a shift in the deprotonation site of 2- and 4-[(2,4,6-trinitrophenyl)amino]benzoic acids (**1** and **2**, respectively), and hence establish the structure (**a** or **b**) of the anion formed in each situation. To this effect, we have employed both the NMR method mentioned above and the theoretical calculation of the energies of the various species.

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† Visiting student from Trinity College, Dublin (Ireland) under the ERASMUS–ECTS Program.

Results

Theoretical calculations

An independent approach to the problem involves the calculation of the energies and structures of the species of interest.[9] Such calculations are generally carried out for the isolated species in the gas phase, which is a major obstacle if solvent effects are to be investigated. A straightforward approach to overcome this difficulty would be to run the calculation for a system comprising a number of solvent molecules such that the solvation shell is adequately represented. However, the increase in the complexity of the system renders this approach impractical. Alternatively, instead of describing the solvent explicitly one can model it as a continuum, with given properties, surrounding the solute molecule.[10] Such models are available both for semiempirical and *ab initio* methods; however, their use is not yet widespread, and some have been so recently introduced that their performance has not yet been fully assessed. Thus, the application of these methods allows us to obtain a completely independent comparison with experimental results, as well as to test their applicability.

The geometry of **1**, **2**, and their anions was firstly optimized with the AM1[11] parameter set in the SPARTAN 3.1[12] implementation. The results are reported in Table 1 as enthalpies of formation (ΔH_f), and as values of ΔH_f relative to the most stable species ($\Delta\Delta H_f$).

The structures of all species have also been optimized with *ab initio* methods; because the main structural features do not greatly differ from those calculated by AM1, we will present only the *ab initio* structures (see below).

Recently, a continuum solvation model for water was devised[13] for the AM1 parameter set (AM1–SM2), in which the solute molecule is enclosed in a solute-shaped cavity, and solute–solvent interaction energies include cavitation and surface tension terms. Thus, as a further step, we calculated the energies of all species in water, using the continuum water model AM1–SM2 in the SPARTAN 3.1 implementation, at the AM1 geometries; the results are also collected in Table 1.

Given the size of the species under investigation, *ab initio* geometry optimizations have been run with the 3-21G basis set[9] at the Hartree–Fock level. This level of theory is known to yield reliable results on both the structure and the relative stability of anions.[9] The results (energies of the various species, and relative energies of the anions) are reported in Table 2. Molecular radii (a_0) for use in SCRF calculations (see below) were also obtained at the same level.

Representative structures thus obtained are depicted in Figs. 1(*a*)–(*c*), and relevant geometrical parameters are in Table 3. In order to minimize their steric repulsion, the two aromatic rings are strongly twisted (dihedral angle 20–40°); the nitro group closest to the other ring is also strongly out of plane.

Geometry optimization of **1a** and **1b** leads to the same structure, in which the O–H (0.982 Å) and N–H (1.838 Å) distances are such that it can be described as **1a** with an intramolecular hydrogen bond (HB). Therefore, at the level of theory employed the oxyanion **1b** is unstable in the gas phase, and the NH proton is transferred to the oxygen giving rise to the nitroanion **1a**. Because only one structure is found for the anion derived from **1**, calculations on this system were not carried on further.

The solvent effect on the deprotonation of **2** was investigated by means of the continuum model known as self-consistent reaction field (SCRF).[14] This model assumes the solute to be placed into a spherical (rather than solute-shaped) cavity of radius a_0, and the continuum to be characterized only by its permittivity ε; the gas-phase Hamiltonian is corrected for the presence of the solvent by a term (R) which depends on μ (solute dipole moment), ε and a_0 according to $R = [2(\varepsilon - 1)/(2\varepsilon + 1)]a_0{}^3\mu$. The calculation of the solvent effect is based only on dipole–dipole interactions, so that specific interactions like hydrogen bonding are not fully accounted for. However, despite its simplicity this method works quite well in many cases;[14] moreover, it is not computationally demanding, and requires only one extra parameter (a_0).

The structures and molecular radii obtained before were employed for the calculation of the energy with the SCRF method (GAUSSIAN 92[15] implementation). The results obtained in DMSO (ε = 46.7) and water (ε = 78.5) are in Table 2.

Table 1 Enthalpies of formation (ΔH_f) and values relative to the most stable species ($\Delta\Delta H_f$) calculated with semiempirical methods

	AM1[a]		AM1-SM2[b]	
Species	ΔH_f/kcal mol^{-1}	$\Delta\Delta H_f$/kcal mol^{-1}	ΔH_f/kcal mol^{-1}	$\Delta\Delta H_f$/kcal mol^{-1}
1	−8.73	—	−22.49	—
1a	−73.64	(2.0)	−121.77	(0.0)
1a closed	−75.61	(0.0)	−121.73	(0.0)
1b	−46.59	29.0	−107.05	14.7
2	−8.56	—	−24.31	—
2a	−75.37	(0.0)	−122.33	(0.0)
2b	−40.46	34.9	−107.25	15.1

[a] AM1 geometry optimization. [b] AM1–SM2 solvation model for water at AM1 geometry.

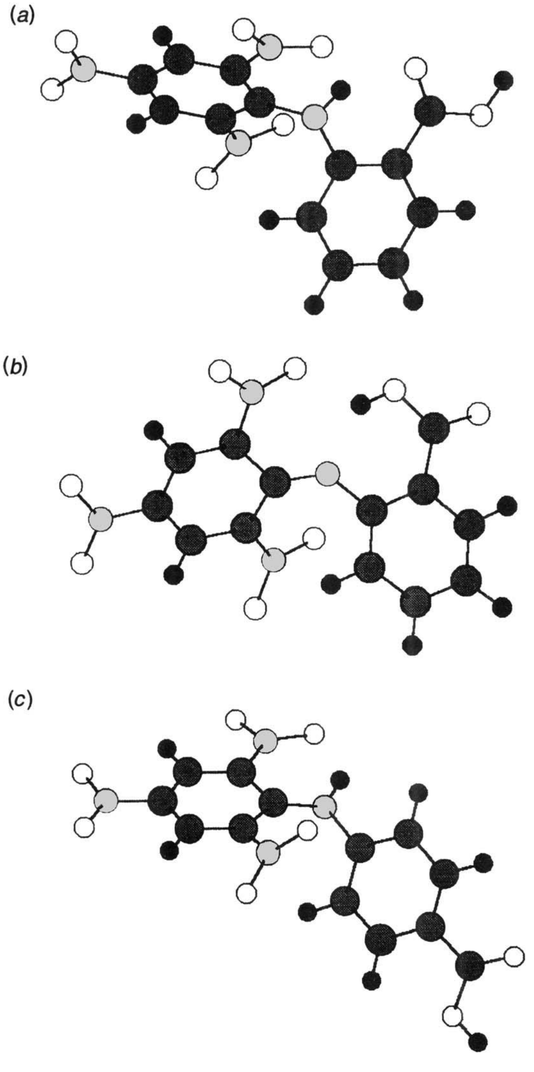


Fig. 1 *Ab initio* HF/3-21G structure of (*a*) 2-[(2,4,6-trinitrophenyl)-amino]benzoic acid **1**; (*b*) anionic form of **1**; (*c*) 4-[(2,4,6-trinitrophenyl)-amino]benzoic acid **2**. C dark grey, H black, N light grey, O white.

Table 2 Absolute (E) and relative (ΔE, kcal mol^{-1}) energies, and molecular radii (a_0) calculated at the HF/3-21G level,[a] and at the SCRF/3-21G level in DMSO and H_2O.[b] 1 au = 627.5 kcal mol^{-1}; 1 Å = 100 pm

	Gas phase			DMSO		H_2O	
Species	E/au	ΔE/kcal mol^{-1}	a_0/Å[c]	E/au	ΔE/kcal mol^{-1}	E/au	ΔE/kcal mol^{-1}
1	−1305.830 695	—	—	—	—	—	—
1a, b	−1305.308 565	—	—	—	—	—	—
2	−1305.831 207	—	5.16	−1305.831 854	—	−1305.831 892	—
2a	−1305.300 635	(0.0)	5.18	−1305.304 162	3.8	−1305.304 234	4.3
2b	−1305.265 090	22.3	5.17	−1305.310 289	(0.0)	−1305.311 092	(0.0)

[a] HF/3-21G geometry optimization. [b] SCRF/3-21G//HF/3-21G. [c] From numerical integration of the molecular volume, and increased by 0.5 Å to allow for the van der Waals radii of surrounding solvent molecules.[14,15] [c] SCRF/3-21G//HF/3-21G.

Table 3 *Ab initio* (HF/3-21G) relevant geometrical parameters

Species	θ(C–C–N–C)/°	α(C–N–C)/°	r(N–H)/Å	r(C=O)/Å	r(C–O)/Å	r(O–H)/Å
1	−28.41	128.38	1.013	1.213	1.349	0.968
1a	−16.25	130.27	1.834	1.212	1.329	0.982
1b	−16.25	130.27	1.838	1.212	1.329	0.982
2	−155.07	129.21	1.000	1.205	1.354	0.968
2a	−165.42	133.19	—	1.211	1.365	0.967
2b	−156.53	129.15	1.001	1.247	1.247	—

NMR Measurements

The NMR spin–lattice relaxation rate ($1/T_1$) of a given nucleus X is affected by the addition or removal of a proton.[8] In the case of spin-$\frac{1}{2}$ nuclei (*e.g.* ^{13}C, ^{15}N, ^{31}P), 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 eqn. (1),[16–18] where S is the nuclear

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

spin quantum number of the nucleus acting as relaxating agent (generally ^1H, $S = \frac{1}{2}$), N is the number of such nuclei located at a distance r_{XH} from the observed nucleus, γ_X and γ_S are the respective magnetogyric ratios, and τ_c is the correlation time. Dipolar relaxation times (T_1^{DD}) lie in the range 10^{-1}–10^2 s, and can be extracted from the overall T_1 by measuring the nuclear Overhauser effect (NOE) η, because $1/T_1^{DD} = (1/T_1)(\eta/\eta_{max})$, where $\eta_{max} = \gamma_H/2\gamma_X$.[17,18] Eqn. (1) shows that the efficiency of dipolar relaxation depends on the number of protons and on the distance r_{XH}. Because of the extremely steep dependence on distance, the nucleus X will be affected essentially only by directly bonded protons. Therefore, protonation of a given nucleus will increase its dipolar relaxation rate, and we expect T_1^{DD} to decrease upon protonation, and to increase upon deprotonation.

Nuclei with $I > \frac{1}{2}$ (*e.g.* ^{14}N, ^{17}O) are very efficiently relaxed by the coupling between their nuclear quadrupole moment and the electric field gradient at the nucleus, and generally T_1 values lie in the 10^{-2}–10^{-4} s range.[16,17] Therefore, the widths of their NMR signals ($W_{\frac{1}{2}}$) are much larger (10–10^3 Hz) than for spin-$\frac{1}{2}$ nuclei (10^{-2}–10^{-1} Hz), and their possible changes following ionization are not easy to predict.

In both cases, the relaxation rates are proportional to τ_c. This essentially depends on the hydrodynamic volume V_m (which may include solvent molecules) and the solution viscosity η; a simplified form is represented by eqn. (2).[17] Therefore, for the

$$\tau_c = \eta V_m/kT \qquad (2)$$

method to work properly, viscosity and temperature must be kept constant among different measurements. A practical consequence of eqn. (2) is that relaxation rates are roughly proportional to the molecular mass of the species, owing to the dependence on V_m. Thus, other things being equal, the relaxation times of **1**, **2** and their ions will be shorter than those of functionally similar compounds with a lower molecular mass.

In conclusion, this method amounts to measuring the T_1 and NOE of all conceivable ionization sites at two values of acid or base strength, where either the neutral or the ionized form is present, keeping the viscosity and temperature constant. In the present case, both atoms at which ionization may take place (O and N) have NMR-active isotopes, *i.e.* ^{17}O, ^{14}N (quadrupolar) and ^{15}N (non-quadrupolar).[16] However, the use of quadrupolar isotopes (^{17}O and ^{14}N) proved unfeasible, because the large molecular mass of **1** and **2** gives rise to very broad signals (see below). The ^{15}N nucleus is not quadrupolar and hence gives narrow, well-resolved lines. However, its use poses several experimental problems[16,19] related to its low natural abundance (0.37%), low negative γ value, and often long T_1 values. We also recall that, owing to the low solubility of the neutrals in DMSO–water, dilute solutions must be employed. Compounds isotopically enriched with ^{15}N were synthesized to overcome these difficulties (see Experimental section). For ^{15}N relaxation measurements according to the method outlined above, the cosolvent water must not be deuteriated, otherwise all exchangeable protons would be rapidly replaced by deuterons with a much lower magnetogyric ratio, so that dipolar relaxation would be very inefficient, because the relative rate depends on $[\gamma(^2H)/\gamma(^1H)]^2 = 0.023$, regardless of the state of ionization of the nitrogen atom. The spectra were obtained in $[^2H_6]$DMSO and in $[^2H_6]$DMSO–H_2O 60:40 w/w (hereafter, the deuteriation suffix will be omitted).

The ^{14}N spectrum of a 0.5 M solution of non-labelled **2** in DMSO showed only the unresolved NO_2 signal at δ −18 ppm. Its linewidth ($W_{\frac{1}{2}}$) of 1.1 kHz is much higher than the typical values for low molecular mass compounds (*e.g.* $W_{\frac{1}{2}}$ = 14 Hz for neat CH_3NO_2).[16] No signal in the amine region (*ca.* −300 ppm) was detected even with 10^5 transients. The electric field gradient at an aniline nitrogen is only slightly higher than in aliphatic amines;[20] therefore this result is mostly due to the higher molecular mass [eqn. (2)].

In DMSO, the ^1H spectrum of the non-labelled acid **1** shows a singlet (δ 11.6 ppm,[21] $W_{\frac{1}{2}}$ = 3 Hz), which disappears upon addition of D_2O. This was assigned to the NH proton by comparison with the ^{15}N spectrum of ^{15}N-**1** (doublet, δ −281.3

ppm, J_{NH} 93). We could not detect the carboxylic proton, which might have been exchanging with the small (but feebly visible) amount of water present in DMSO. The ^1H spectrum of the potassium salt shows a broad signal at δ 14.8 ppm, whose assignment is not straightforward because the appearance is also the same with the labelled salt. This low shielding is typical of hydrogen-bonded protons, but lies short of the range for low-barrier HBs, (16–20 ppm).[22] In fact, deprotonation at either site yields an anion which can be stabilized by an intramolecular hydrogen bond, as revealed by the calculations. In such a structure the remaining acidic proton would be in an intermediate situation between **1a** and **1b**, so that exchange between the two sites, or with traces of water, would account for its linewidth. ^{15}N T_1 and NOE values are in Table 4. The overall change of T_1^{DD} between neutral and anionic form is small, which seemingly indicates that deprotonation at nitrogen is not occurring. This result is in contrast with all available data, which indicate that the nitrogen atom is the most acidic site in DMSO. However, theoretical calculations and NMR data agree in indicating that an intramolecular hydrogen bond exists in **1a**, where the N–H distance is small enough to affect the relaxation of nitrogen. In any event, because these results do not allow for a clear-cut assignment of the ionization site even in the reference solvent, we did not proceed with the measurements in DMSO–H_2O.

For acid **2**, the general features of ^1H and ^{15}N spectra are quite similar to those of **1**. Of course, while in DMSO one can detect the acidic protons, in DMSO–H_2O both exchange with water, and no interesting feature remains in the ^1H spectra. The results obtained both in DMSO and DMSO–H_2O 60:40 are also reported in Table 4.

Discussion

Theoretical calculations

AM1 calculations predict *N*-deprotonation to be favoured over *O*-deprotonation by *ca.* 30 kcal mol^{-1} for both **1** and **2**. This is not unexpected, because the ion that can best delocalize its charge (**a**) is favoured in the gas phase. In water (as modelled by the AM1-SM2 method), *N*-deprotonation is favoured by a lower amount of energy (15 kcal mol^{-1}), owing to the more exothermic enthalpy of hydration of the oxyanion **b** ($\Delta H_f^{AM1\text{-}SM2} - \Delta H_f^{AM1}$), but the overall energy balance is not shifted. From these calculations it can be concluded that the nitrogen atom is intrinsically the most acidic site both in the gas phase and water, although in the latter phase the stability difference between the two anions is diminished.

At the *ab initio* level, **2a** is again favoured over **2b** (by 22 kcal mol^{-1}). Modelling the solvent by the SCRF method, the oxyanion **2b** becomes the most stable, albeit by only *ca.* 4 kcal mol^{-1}, both in water and DMSO. However, this method does not differentiate the two solvents; this could be expected on the basis of their high ε values, which lead to similar reaction fields [$2(\varepsilon - 1)/(2\varepsilon + 1) = 0.98$ and 0.97 for water and DMSO, respectively]. Hence, introducing any solvent with a high relative permittivity stabilizes the ion **2b** to the point that it becomes the most stable one. Although the trend is correct, these results do not furnish a clear-cut answer to the question sought, because the solvent effect specifically exerted by H_2O and DMSO is not differentiated, which calls for an experimental evaluation.

In any event, SCRF appears superior to AM1-SM2, and proves rather successful even in this difficult case (because the energy balance was reversed even with very different gas-phase energies). The poorer performance of the latter method might actually be due to limitations of the underlying quantum mechanical (semiempirical) method rather than to the treatment of the solvent. The two treatments can however be mixed, *i.e.* one can use the *ab initio* energies (which better describe the isolated molecule) and AM1-SM2 solvation energies (which supposedly better describe the solvent).[13] For example, we can recalculate the stability difference between **2a** and **2b**; in this way, **2a** is still more stable by 2.5 kcal mol^{-1}. Hence, even with this mixed treatment one does not obtain the inversion of stability calculated by SCRF alone, although the energy balance is now similar. In conclusion, SCRF still seems superior, despite the rougher approximations it is based on.

Table 4 ^{15}N chemical shifts, T_1s and NOEs in [$^2H_6$]DMSO and [$^2H_6$]DMSO–H_2O 60:40[a]

Species	δ[b]	T_1/s	η[c]	T_1^{DD}/s
Solvent: $(CD_3)_2SO$				
1[d]	−281.3	1.22	−6.5	1.22
1 anion	−316.1	1.21	−4.3	1.40
2[d]	−280.6	1.62	−5.5	1.62
2 anion	−314.6	4.18	−4.5	4.57
Solvent: $(CD_3)_2SO$–H_2O 60/40				
2[d]	−280.2	1.39	−6.0	1.39
2 anion[e]	−320.5	1.83	−5.6	1.83

[a] In [$^2H_6$]DMSO (0.5 M) or [$^2H_6$]–H_2O 60:40 (0.05 M because of the low solubility of the neutrals); anions introduced as potassium salts. [b] Relative to external CH_3NO_2. [c] Values for the neutrals are higher than the theoretical maximum (−4.93); this is probably due to an underestimation of the intensities of the reference spectra, due in turn to the low signal/noise ratio; T_1^{DD} is assumed equal to T_1. [d] Run with a few equivalents of CF_3COOH added to suppress dissociation. [e] Run in a solution containing 0.01 M NaOH to prevent protonation of the anion by the solvent.

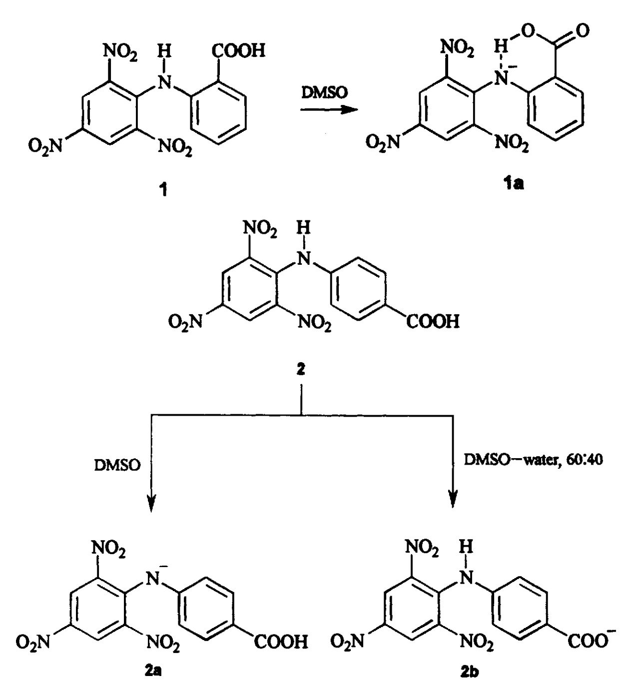
^{15}N NMR Measurements

There is no appreciable T_1 change for **1** on going from neutral to anionic form. Because there is no reasonable doubt that ionization takes place at nitrogen in DMSO, as confirmed by the calculations, one can conclude that ionization does take place at nitrogen, but the structure of the anion thus formed is such that the magnetic environment of the nitrogen (and hence its relaxation rate) is not markedly perturbed, and therefore whether deprotonation of **1** takes place at N or O cannot be answered by NMR relaxation data. However, we note that, if the acidity of NH and OH were very similar, the conditions for a low-barrier HB would apply and the HB proton would resonate at δ = 16–20 ppm,[22] which is not found.

In the case of **2**, there is a marked increase of T_1^{DD} on going from neutral to anionic form in DMSO, whereas in DMSO–H_2O T_1^{DD} remains essentially constant, which indicates that in the anion there is an N–H bond.

At this point, we wish to emphasize that an analysis of the trend followed by ^{15}N chemical shifts for neutral and anionic species gives no information on this problem. In fact, the deprotonation of aryl nitrogen acids (like $PhNH_2$ and Ph_2NH) is known to cause a deshielding by 60–80 ppm,[23] whereas the 4-aminobenzoate anion ($H_2NC_6H_4CO_2^-$)[24] is slightly shielded (by 8 ppm) with respect to the neutral compound. However, in our case the observed change is a shielding of similar magnitude in all cases ($\Delta\delta$ −34.8 for **1** in DMSO, −34.0 for **2** in DMSO, and −40.3 for **2** in DMSO–H_2O). In the last instance, the variation is slightly higher in magnitude, but has the same sign as the others, and is probably due to a solvent effect. Therefore this approach does not differentiate between the two situations, while relaxation rates do.

In summary, deprotonation of **1** in DMSO leads to a nitroanion stabilized by the OH proton through an intramolecular HB, whereas **2** is a nitrogen acid in DMSO and an oxygen acid in DMSO–H_2O (Scheme 2). The reason for this behaviour lies in the competition between intrinsic acidity and solvation. Oxyanions are more strongly hydrated than nitroanions,[3,25–27] but NH acidity is enhanced (at the expense of an even weaker solvation of (**a**) by its extended charge delocalization. Thus, the relative stability of **a** and **b** reverses according to the different solvating properties of water and



Scheme 2

DMSO. Therefore, the same structural change (introduction of electron-withdrawing groups) has two contrasting effects on acid strength, *i.e.* an increase due to a better charge delocalization and possibly a decrease due to a less favourable solvation, the balance of which is not straightforward to predict. Similar effects have been found for acid–base equilibria in concentrated acid[2,28] and basic[27] systems.

Experimental

Preparation of 1 and 2

^{15}N-Labelled **1** and **2** were prepared from commercial [^{15}N]-2-aminobenzoic acid (99% ^{15}N, CIL) or [^{15}N]-4-aminobenzoic acid (see preparation below) and 2,4,6-trinitrochlorobenzene in ethanol[29] (50–70% yield) and purified by flash chromatography.

The following synthetic routes for preparing 4-aminobenzoic acid were tested and rejected. (*a*) Diazotization of ethyl 4-aminobenzoate followed by a Sandmeyer reaction with NO_2^-/Cu was unsuccessful because of the low yield of the latter step. (*b*) Nitration of toluene with HNO_3/H_2SO_4 or HNO_3/Ac_2O is hampered by the fact that concentrated commercial $H^{15}NO_3$ (40%, 8 M) is not suitable for nitration, which in any event yields mostly 2-nitrotoluene. (*c*) Carboxylation of acetanilide with $(COCl)_2/AlCl_3$ under typical conditions failed to yield any carboxylated product.

^{15}N-4-nitrotoluene was prepared from the nitration of 4-bis(trifluoroacetato)thallium toluene, in turn prepared *in situ* from toluene and $Tl(CF_3COO)_3$.[30] In a 50 ml round-bottomed flask fitted with a $CaCl_2$ tube, containing a solution of 1.84 g (3.35 mmol) of $Tl(CF_3COO)_3$ in 10 ml of CF_3COOH, toluene (0.36 g, 3.35 mmol) is added. After a few minutes a white precipitate of $4\text{-}CH_3C_6H_4Tl(CF_3COO)_2$ forms, and the mixture is stirred for 30 min. The flask is then cooled in ice, and 0.71 g (11 mmol) of $Na^{15}NO_2$ (99% ^{15}N, CIL)‡ are added. The solution darkens immediately, and NO_2 is evolved; the mixture is stirred at 30 °C for 12 h, whereby a light-yellow solid separates. The reaction mixture is then poured into water and extracted with benzene. The dried extracts yield crude ^{15}N-4-nitrotoluene as a dark solid, which is purified by flash chromatography with 4:1 Et_2O/EtOAc (71% yield). The product was subsequently oxidized to ^{15}N-4-nitrobenzoic acid (65% yield) with $K_2Cr_2O_7/H_2SO_4$. ^{15}N-4-Nitrobenzoic acid was eventually reduced to ^{15}N-4-aminobenzoic acid in 88% yield with $SnCl_2{\cdot}H_2O$.[31]

Potassium salts of **1** and **2** were prepared by suspending the acids in an aqueous solution of the stoichiometric amount of $KHCO_3$ at 70 °C for 1 h and evaporating the solvent. Conversion to the salt was easily monitored by the colour change (from bright yellow to dark red).

NMR Measurements

NMR spectra were run at 25 °C on Bruker AC 200 (4.7 T, ^{15}N 20.26 MHz) or AM 400 (9.4 T, ^{14}N 28.89 MHz, ^{15}N 40.53 MHz) instruments with 5 or 10 mm broadband probes. The samples were degassed by three freeze–pump–thaw cycles. ^{14}N and ^{15}N chemical shifts are referred to external neat nitromethane. Typical acquisition parameters for ^{15}N (AM 400) were: spectral window 3 kHz, $\pi/2$ pulse length (12.5 µs), 16 K data points in acquisition. T_1s were measured by saturation-recovery, and NOEs by non-selective proton irradiation, with an irradiation time of four to five times the previously determined T_1.

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‡ The sample was stated to contain 10% $Na^{15}NO_3$, which did not adversely affect the reaction.

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