Hydrogen isotope fractionation factors for *N*,*N*-dimethylbenzylammonium ion and some related species: an unusually strong preference for deuterium over protium

Hong-Xun Guo and A. Jerry Kresge *

[a] *Department of Chemistry, University of Toronto, Toronto, Ontario M5S 3H6, Canada*

Deuterium fractionation factors were determined by the ^{1}H and ^{13}C NMR methods in aqueous solution for $PhCH_2NLMe_2^+$ (φ = 1.47 ± 0.05), $PhCH_2OL$ (φ = 1.04 ± 0.06), $PhCO_2L$ (φ = 1.04 ± 0.08), and CH_3CO_2L (φ = 0.99 ± 0.02). The medium effect for transferring $PhCH_2NMe_2$ from H_2O to D_2O, Φ = 1.025 ± 0.003, was also determined by partitioning this substance between water and immiscible organic solvents, and a UV spectroscopic method was used to measure the solvent isotope effect on the acid ionization of $PhCH_2NLMe_2^+$, $(Q_a)_H/(Q_a)_D$ = 4.88 ± 0.16. This solvent isotope effect agrees well with the value predicted using the relevant fractionation factors, $(Q_a)_H/(Q_a)_D$ = 4.38 ± 0.28. The unusually large value of φ for $PhCH_2NLMe_2^+$ is attributed to stiffened bending vibrations of its N–L bond imposed by the tetrahedral structure of the ion and the bulk of its methyl groups.

Deuterium fractionation factors have proved to be especially useful in analysing and predicting solvent isotope effects in light (H_2O) and heavy (D_2O) water.[1] Such fractionation factors are partial isotope effects attributable to individual hydrogens. They are expressed as D : H ratios and are defined in terms of isotope exchange equilibria involving the solvent: the fractionation factor at some particular hydrogenic site, φ_i, is equal to the D : H ratio at that site, $(D/H)_i$, compared to the D : H ratio of the solvent with which it is equilibrated, $(D/H)_s$, eqn. (1).

$$\varphi_i = (D/H)_i/(D/H)_s \quad (1)$$

Fractionation factors reflect the tightness of bonding to the hydrogens they represent: $\varphi > 1$ denotes tighter, and $\varphi < 1$ looser, bonding than that in the reference oxygen–hydrogen bonds of water. For example, the less than unit value of the fractionation factor for the hydronium ion in aqueous solution, $\varphi = l\dagger = 0.69$,[1,2] indicates that the bonding of the hydrogens in this substance is looser than that of the hydrogens in water, which is consistent with the lower vibrational frequencies of the hydronium ion than those of water.[3] This looser bonding is believed to be a consequence of the positive charge of the hydronium ion which draws electron density out of the bonds of this substance, making them weaker.

It is generally believed that this difference in fractionation factor between hydronium ion and water extends to all positively charged and neutral oxygen–hydrogen bonds. A similar difference, however, appears not to apply to positively charged and neutral nitrogen–hydrogen bonds: we have recently shown that the fractionation factor for the positively charged N–L‡ bonds of benzylammonium ion, **1**, φ = 1.08, is actually greater

$PhCH_2NL_3^+$ **1** $PhCH_2NL_2$ **2** $PhCH_2NLMe_2^+$ **3**

than that for the neutral N–L bonds of benzylamine, **2**, φ = 0.96.[4] Examination of vibrational frequencies of NL_4^+ and NL_3 as surrogates for $PhCH_2NL_3^+$ and $PhCH_2NL_2$ showed that the expected bond weakening accompanying positive charge introduction was indeed reflected in lowered stretching vibration frequencies, but this effect was offset by compensatory increases in bending vibration frequencies. These increases were attributed to the tetrahedral structure of ammonium ions which restricts N–L bending motion in these substances. The hydronium ion, on the other hand, is trigonal with a very flat pyramidal structure that allows considerably less restricted 'out-of-plane' bending motion. The lowered stretching frequencies of the hydronium ion are consequently not offset by increased bending frequencies, and its fractionation factor is therefore less than that of water.

It follows from this hypothesis that replacement of the hydrogens of an ammonium ion with larger groups should restrict bending motion even more and lead to still greater fractionation factors. We have therefore examined the *N*,*N*-dimethylbenzylammonium ion, **3**, in order to see whether or not this is so. We determined the fractionation factor for this substance by the traditional ^{1}H NMR method[2] and also by the more recently devised ^{13}C NMR method.[5] We made the ^{13}C NMR measurements using substrate enriched in ^{13}C at the benzylic position, which we prepared from [α-^{13}C]benzoic acid; we therefore also determined the fractionation factor for benzoic acid, its reduction product benzyl alcohol and acetic acid as well.

Experimental

Materials

[α-^{13}C]*N*,*N*-Dimethylbenzylamine was prepared by lithium aluminium hydride reduction of [α-^{13}C]*N*,*N*-dimethylbenzamide, itself obtained from [α-^{13}C]benzoic acid (Isotec, Inc. 99 atom% ^{13}C at the labelled position) *via* the acid chloride. [α-^{13}C]Benzyl alcohol was prepared by lithium aluminium hydride reduction of the same benzoic acid. [α-^{13}C]Methyltrimethylammonium iodide was prepared previously.[4] The identities of these substances were confirmed by their ^{1}H NMR spectra. All other materials were best available commercial grades.

NMR

NMR measurements were made with Varian Gemini 200 or Gemini 300 instruments operating at probe temperatures of 25 °C. Coaxial 5 mm sample tubes were used, with the sample solution containing substrate plus the tetramethylammonium iodide (0.001 M) reference placed in the outer compartment and D_2O (to provide a frequency lock) placed in the inner compartment. Because the reference and substrate were present

† The special symbol '*l*' is generally used for the fractionation factor of the hydronium ion.

‡ The symbol 'L' denotes either protium or deuterium, *i.e.* L = H or D.

together in the same solution, no correction for differences in magnetic susceptibility of the H_2O, HDO and D_2O solvents were necessary.

pQ_a determination

The acid dissociation constant, pQ_a,§ of *N*,*N*-dimethylbenzylammonium ion was determined spectrophotometrically using the change in absorbance at λ 224 nm that accompanies this ionization reaction. Measurements were made with a Cary 118 spectrometer, operating with the cell compartment at 25.0 ± 0.05 °C, on solutions of fixed stoichiometric *N*,*N*-dimethylbenzylammonium ion concentrations (*ca.* 5.5×10^{-5} M) but variable, and known, hydrogen ion concentrations; ionic strength was maintained at 0.10 M. Equilibrium constants, Q_a, were evaluated by non-linear least-squares fitting of eqn. (2), in

$$A_{obs} = \frac{A_B Q_a + A_{BH}[L^+]}{Q_a + [L^+]} \quad (2)$$

which A_B and A_{BH} are absorbances of the substrate completely in its basic and acidic forms respectively and $[L^+]$ is hydrogen ion concentration.

Partition coefficients

Partition coefficients for the distribution of *N*,*N*-dimethylbenzylamine between organic solvents and water were determined spectrophotometrically using the absorbance of the amine at λ 260 nm. Measurements were made with a Cary 118 spectrometer whose cell compartment was thermostatted at 25.0 ± 0.05 °C. The absorbance of aqueous solutions of the amine (*ca.* 1×10^{-3} M) was first measured; 5.00 ml aliquots of these solutions were then shaken vigorously with 5.00 ml volumes of organic solvent, the resulting mixtures were kept in a 25.0 ± 0.05 °C bath for 4–5 h to achieve phase separation, and the absorbance of the organic layer was then measured again. Partition coefficients, *K*, were calculated using eqn. (3) in which

$$K = \frac{A_0}{A_0 - A_{eq}} \quad (3)$$

A_0 and A_{eq} are absorbances of the aqueous solutions before and after equilibration with the organic solvent.

Results

Fractionation factors

Amines and ammonium ions dissolved in water exchange the hydrogens of their N–L bonds with the solvent rapidly, and this makes it difficult to remove these substances from solution in an H_2O–D_2O mixture for conventional isotopic analysis without disturbing their deuterium content. Fortunately, methods have been developed for conducting the isotopic assay directly on the dissolved, rapidly exchanging substances. The ^{13}C NMR method is based upon the fact that substitution of deuterium for protium in a bond to hydrogen produces an isotope effect on the chemical shift of nearby carbon atoms. In a rapidly exchanging system, the change in chemical shift caused by this isotope effect will depend upon the deuterium to protium ratio in the isotopically substituted bond, and the phenomena can consequently be used to report the average deuterium content of that bond.

Use of this method requires measurement of the ^{13}C chemical shift of the reporting carbon atom under three different conditions: in H_2O solution (δ_H), in D_2O solution (δ_D) and in an H_2O–D_2O mixture of deuterium atom fraction *x* (δ_x). The deuterium to protium ratio at the exchanging site is then equal to $(\delta_H - \delta_x)/(\delta_x - \delta_D)$, and the fractionation factor at that site is equal to this ratio divided by the deuterium to protium ratio of the solvent, as shown in eqn. (4).

$$\varphi = \left(\frac{\delta_H - \delta_x}{\delta_x - \delta_H}\right) \Big/ \frac{x}{1 - x} \quad (4)$$

Observed ^{13}C NMR chemical shifts of the enriched α-carbon atom of *N*,*N*-dimethylbenzylammonium ion proved to be linearly dependent on the concentration of this substance in all three solvents employed, H_2O, D_2O and a 50:50 H_2O–D_2O mixture. Measurements were consequently made over a range of concentrations, at a fixed ionic strength (0.10 M, $NaClO_4$), and the data were extrapolated to zero concentration by linear least-squares analysis. An example is shown in Fig. 1. The zero-concentration chemical shifts were then used to calculate the fractionation factors listed in Table 1. Similar measurements were made on benzyl alcohol, benzoic acid and acetic acid and the fractionation factors obtained for these substances are also listed in Table 1.

Another method of determining fractionation factors for rapidly exchanging hydrogens uses ^{1}H NMR. This method is based upon the fact that the ^{1}H NMR signal for hydrogens exchanging rapidly with the solvent is a composite line whose position depends upon the relative amounts of protium in the solvent and at the exchanging site. Introduction of deuterium into the system will change these relative amounts (unless the fractionation factor is unity), and that will change the position of the NMR signal. This change in position, plus knowledge of the stoichiometry of the system, may then be used to calculate the fractionation factor.

The relationship governing this phenomenon for a single sol-

Table 1 Summary of fractionation factors[a]

Species	φ		
	^{13}C NMR[b]	^{1}H NMR[b]	Weighted average
$PhCH_2NLMe_2^+$	1.482 ± 0.058	1.461 ± 0.076	1.474 ± 0.046
$PhCH_2OL$	1.102 ± 0.115	1.024 ± 0.065	1.043 ± 0.056
$PhCO_2L$	1.063 ± 0.129	1.031 ± 0.107	1.044 ± 0.082
CH_3CO_2L	0.990 ± 0.130	0.981 ± 0.067	0.986 ± 0.022
	0.990 ± 0.067	0.985 ± 0.026	

[a] In aqueous solution, 25 °C, ionic strength = 0.10 M. [b] Uncertainties are standard deviations obtained by propagating errors in constituent NMR parameters.

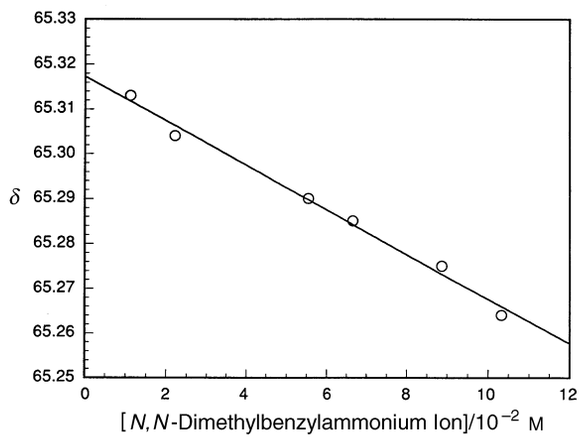

Fig. 1 Dependence of the chemical shift of the α-carbon atom of *N*,*N*-dimethylbenzylammonium ion on the concentration of this ion in H_2O solution

§ The acid dissociation constants determined here are concentration quotients applicable at the ionic strength of the measurements (0.10 M). The symbol 'Q_a' is used for such concentration quotients and 'K_a' is reserved for thermodynamic acidity constants referred to zero ionic strength.

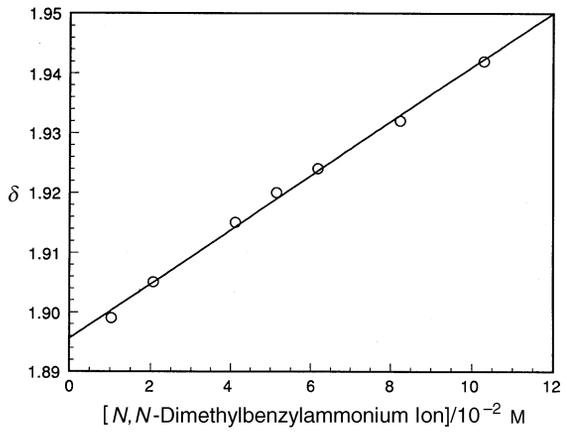

Fig. 2 Relationship between *N*,*N*-dimethylbenzylammonium ion concentration and the composite chemical shift of this ion and water in H_2O solution; chemical shifts are referenced to the methyl group signal of the ion

ute is given in eqn. (5),[1b,2,6] where δ_0 is the chemical shift of the

$$\delta_{obs} = \delta_0 + \frac{\delta_s[S]}{1 - x + x\varphi} \quad (5)$$

solvent and δ_s is that of the solute. The observed chemical shift, δ_{obs}, is thus expected to be a linear function of solute concentration. This was found to be so for all of the substrates examined here; Fig. 2 shows a typical example. The slope of the linear relationship in H_2O, where $x = 0$, provides δ_s, and that, in combination with the slope determined in an H_2O–D_2O mixture of atom fraction x, then affords φ.

This method was used to determine fractionation factors for each of the four substrates to which the ^{13}C NMR method had been applied; the results obtained are also listed in Table 1. It may be seen that the fractionation factors determined by the two methods agree with each other very well: the differences between the results obtained by the two methods are all well within the combined uncertainties of the individual determinations. This is significant, because fractionation factors determined by the ^1H NMR method include medium effects whereas those determined by the ^{13}C NMR method do not;[4] medium effects in the present systems are thus evidently too small to be detected by the methods employed.

Medium effects are produced by water molecules in the solvation shells of the substances to which they refer. These water molecules are different from bulk water, and their fractionation factors are consequently different from the unit value that applies to bulk water. The difference is usually quite small, but because the number of solvating water molecules is appreciable, their combined influence could be significant.

pQ_a

The solvent isotope effect on the acid ionization of *N*,*N*-dimethylbenzylammonium ion, eqn. (6), can be used to provide

$$PhCH_2NLMe_2^+ + L_2O \overset{Q_a}{\rightleftharpoons} PhCH_2NMe_2 + L_3O^+ \quad (6)$$

a check on the reliability of the presently determined fractionation factor for this substance. This isotope effect may be expressed in terms of fractionation factors for all of the substances taking part in this reaction, as shown in eqn. (7). In this expression, $\varphi_{\mathbf{PhCH_2NLMe_2^+}}$ and l are individual site fractionation factors for single hydrogens in $PhCH_2NLMe_2^+$ and L_3O^+, and l is raised to the third power because there are three hydrogens in L_3O^+, each with the fractionation factor l. The conjugate base $PhCH_2NMe_2$ has no exchangeable hydrogens, but it may contribute a medium effect and the term $\Phi_{\mathbf{PhCH_2NMe_2}}$ is therefore included. (Following Albery,[7] we use lower case φ for individual site fractionation factors and upper case Φ for medium effects.) The fractionation factor for L_2O is unity, and the term for this substance therefore drops out of eqn. (7).

$$\frac{(Q_a)_H}{(Q_a)_D} = \frac{\varphi_{\mathbf{PhCH_2NLMe_2^+}}}{\Phi_{\mathbf{PhCH_2NMe_2}}\, l^3} \quad (7)$$

Table 2 Partition coefficients for distribution of *N*,*N*-dimethylbenzylamine between water and organic solvents[a]

Organic solvent	*K*		
	H_2O	D_2O	Φ
Hexane	0.1144	0.1163	1.016
	0.1136	0.1169	1.029
Cyclohexane	0.1161	0.1195	1.029
	0.1169	0.1193	1.020
Heptane	0.08758	0.09041	1.032
	0.08850	0.09041	1.022
	Average: $\Phi = 1.025 \pm 0.003$		

[a] At 25 °C; ionic strength of aqueous phase = 0.10 M.

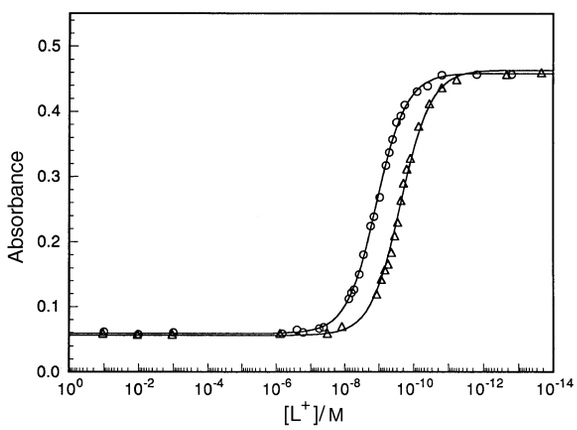

Fig. 3 Titration curves for the ionization of *N*,*N*-dimethylbenzylammonium ion in H_2O (O) and D_2O (Δ)

Medium effects can be evaluated as solvent isotope effects on partition coefficients for distribution of substrates between water and an immiscible organic solvent. Such isotope effects were determined for *N*,*N*-dimethylbenzylamine. The results are summarized in Table 2; they lead to the medium effect $\Phi = 1.025 \pm 0.003$. This medium effect is quite small, which is consistent with our inability to determine medium effects as differences between fractionation factors obtained by the ^1H and ^{13}C NMR methods as discussed above.

We determined the acid ionization constant of *N*,*N*-dimethylbenzylammonium ion in H_2O and in D_2O by making absorbance measurements in perchloric acid and sodium hydroxide solutions as well as in biphosphate ion, boric acid and hydrogencarbonate ion buffers. Ionic strength was maintained at 0.10 M by adding $NaClO_4$ as required. Hydrogen ion concentrations of the buffers were obtained by calculation, using $pK_a(H_2O) = 7.201$ and $K_a(H_2O)/K_a(D_2O) = 3.43$ for biphosphate ion,[8] $pK_a(H_2O) = 9.223$ and $K(H_2O)/K(D_2O) = 3.28$ for boric acid[9] and $pK_a = 10.329$ and $K(H_2O)/K(D_2O) = 5.07$ for hydrogencarbonate ion,[10] plus activity coefficients recommended by Bates;[11] the isotope effect on the autoprotolysis of water was taken to be $K(H_2O)/K(D_2O) = 7.28$.[12]

As Fig. 3 illustrates, the results gave well-defined titration curves. Two sets of measurements were made in H_2O and two in D_2O. Least-squares fitting of eqn. (2) gave $Q_a = (1.172 \pm 0.026) \times 10^{-9}$ and $(1.112 \pm 0.028) \times 10^{-9}$ M for H_2O and

$Q_a = (2.316 \pm 0.086) \times 10^{-10}$ and $(2.387 \pm 0.101) \times 10^{-10}$ M for D_2O. The weighted averages of these values are $Q_a(H_2O) = (1.144 \pm 0.019) \times 10^{-9}$ M and $Q_a(D_2O) = (2.346 \pm 0.066) \times 10^{-10}$ M, and the ratio of these averages gives the solvent isotope effect $Q_a(H_2O)/Q_a(D_2O) = 4.88 \pm 0.16$. The value for H_2O may be converted into a thermodynamic acidity constant by application of appropriate activity coefficients;[11] the result gives $pK_a = 8.926 \pm 0.007$, in good agreement with $pK_a = 8.91$ reported in a previous study.[13]

Evaluation of eqn. (7) using the values of $\varphi_{PhCH_2NLMe_2^+}$ and $\Phi_{PhCH_2NMe_2}$ determined here plus $l = 0.69 \pm 0.01$[2] gives the isotope effect 4.38 ± 0.28. This agrees well with the directly measured result 4.88 ± 0.16, and that adds confidence to the reliability of both the fractionation factor and acid ionization constant determinations.

Discussion

The fractionation factor determined here for *N*,*N*-dimethylbenzylammonium ion, $\varphi_{PhCH_2NLMe_2^+} = 1.47 \pm 0.05$, is considerably greater than that for benzylammonium ion, $\varphi_{PhCH_2NL_3} = 1.08 \pm 0.02$.[4] This difference supports the hypothesis that the tetrahedral structure of ammonium ions restricts the bending motion of their N–L bonds and the consequent stiffening of these bonds raises their fractionation factors. Because methyl groups are larger than hydrogen, this bond stiffening should be greater for $PhCH_2NLMe_2^+$ than for $PhCH_2NL_3^+$ and the fractionation factor for the former should be greater than that for the latter, as observed.

The presently determined fractionation factor for $PhCH_2NLMe_2^+$ is in fact unusually large. Values of the magnitude of the present result have been reported recently for some of the backbone amide hydrogens of staphylococcal nuclease,[14] but fractionation factors much greater than unity are rare. The only value for an ammonium ion derived from a tertiary amine of which we are aware is $\varphi = 1.23$ for *N*,*N*-dimethyl-*p*-nitroanilinium ion;[15] though not as great as our result, this fractionation factor is large, and that offers further support for the idea that bending vibrations play an important role in determining the magnitude of fractionation factors of ammonium ions.

None of the fractionation factors determined here for the oxygen–hydrogen bonds of $PhCH_2OL$, $PhCO_2L$ and CH_3CO_2L is significantly different from unity (see Table 1), and that for CH_3CO_2L, $\varphi = 0.99 \pm 0.02$, agrees well with $\varphi = 0.96 \pm 0.02$ reported for this substance before.[9] This provides support for the general belief that fractionation factors for all neutral O–L bonds are similar and close to the unity value of bulk water.

It is interesting that the fractionation factors for $PhCH_2OL$ and $PhCO_2L$ are similar despite the great difference in acidity of these two substances. It was once believed that solvent isotope effects on the ionization of acids increase with decreasing strength of the acid, an effect that should be reflected in a tendency for fractionation factors of the acids to increase with decreasing acid strength. With the accumulation of more evidence, however, this idea was found to be incorrect.[16]

Acknowledgements

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