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Position-Specific Secondary Deuterium Isotope Effects on Basicity of Pyridine

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Abstract: Secondary isotope effects (IEs) on basicities of various deuterated pyridine isotopologues and of 2,6-lutidine-2,6-$(CD_3)_2$ have been accurately measured in aqueous solution by an NMR titration method applicable to a mixture. Deuteration at any position of pyridine increases the basicity, but the IE per deuterium is largest for substitution at the 3-position and smallest for the 2-position, which is closest to the site of *N*-protonation, smaller even than that for 2-CD_3 substitution. Computations at the B3LYP/cc-pVTZ level overestimate the magnitude of the measured IEs but largely reproduce the variability with isotopic position. Because the calculated IEs are based on changes in vibrational frequencies on *N*-protonation, the correspondence between calculated and experimental IEs implies that they arise from zero-point energies, rather than from inductive effects.

Introduction

Isotope effects (IEs) are informative features of rates and equilibria.[1] In contrast to primary IEs, where a bond to the isotope is broken, in the case of secondary IEs that bond remains intact. Kinetic IEs continue to provide valuable insight into mechanisms of organic,[2] organometallic,[3] and enzyme-catalyzed reactions.[4] Equilibrium IEs provide subtle details regarding conformational preferences and intermolecular interactions.[5] The topic of secondary equilibrium IEs has been reviewed recently.[6]

Recently we have verified that secondary deuterium IEs increase the basicity of a wide variety of amines.[7] Similarly, acidities of carboxylic acids and phenols are decreased detectably by deuterium substitution, even at remote positions.[8] For aliphatic acids the magnitudes of the IEs diminish as the deuterium becomes more distant from the OH, as expected. Yet the magnitudes of the IEs in both phenol and benzoic acid do not decrease as the deuterium moves from ortho to meta to para. The data are supported by *ab initio* computations that relate the IEs to changes of vibrational frequencies and zero-point energies. However, the experimental IEs are considerably smaller than the calculated ones, a discrepancy that is likely to be due to solvation. Except for the simple case of formate anion, it is difficult to account for the IEs in terms of electron delocalization that lowers vibrational frequencies. To probe the generality of a nearly constant IE upon deuteration at ortho, meta, and para positions, we now address secondary deuterium IEs on the basicity of pyridines.

According to UV spectrophotometric measurements in aqueous buffers, pyridine-d_5 is more basic than pyridine, with K_a^H/K_a^D equal to 1.048 ± 0.028, corresponding to a ΔpK_a of 0.02.[9] However, a subsequent report, using the same method, gave a ΔpK_a of 0.11 ± 0.015 at 293 K.[10] The substantial disagreement can be ascribed to difficulty in making the measurements, owing to interference from the vibrational structure present on the UV absorbances, which varies not only between pyridine and pyridinium ion but also between isotopologues. More recently, the gas-phase equilibrium constant for proton transfer from pyridinium ion to pyridine-d_5 was found to be 1.24 ± 0.06 at

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331 K,[11] corresponding to a ΔpK_a of 0.09. This agrees well with the second value in aqueous solution and also with the IE derived from vibrational frequencies calculated at the B3LYP/cc-pVTZ level. However, the agreement with the solution ΔpK_a may be fortuitous.

We therefore have undertaken to resolve the disagreement between the two measurements in aqueous solution. This topic is of further interest because of the recent discovery of a low-temperature polymorph of pyridine-d_5, different from that of pyridine-h_5.[12] Also, all the previous IE studies addressed only pyridine-d_5, and the errors of ± 0.02 precluded measurement of the small contributions of individual deuteriums. It would be informative to distinguish the separate contributions from the various positions around the ring as well as to assess the IE of CD_3 groups in 2,6-lutidine (2,6-dimethylpyridine).

NMR titration can measure relative basicities with great precision.[13] When successive small aliquots of acid are added to a mixture of bases, the one that is more basic is preferentially protonated. Its chemical shift thus moves ahead of that of the less basic one. The acidity constants K_a and chemical shifts δ can then be related through eq 1, where δ^0 or δ^+ is the shift for the deprotonated or protonated form, respectively, as measured at the beginning or end of the titration. Therefore a plot of the quantity on the left vs $(\delta_H - \delta_H{}^0)(\delta_D{}^+ - \delta_D)$ should be linear with a zero intercept and with a slope equal to the ratio of acidity constants. Any reporter nucleus can be utilized for this purpose, but ^{13}C is most suitable because its chemical shifts are quite sensitive to the state of pyridine protonation and because there are several nuclei in the molecule that provide independent measurements of $K_a{}^H/K_a{}^D$.

$$(\delta_H^+ - \delta_H)(\delta_D - \delta_D^0) = (K_a^H/K_a^D)(\delta_H - \delta_H^0)(\delta_D^+ - \delta_D) \quad (1)$$

We now report highly accurate position-specific secondary deuterium IEs on the basicities of pyridine and 2,6-lutidine. We have also modeled the IEs by *ab initio* computations. In all cases deuterium substitution increases basicity, and we attribute the IEs to changes of vibrational frequencies on *N*-protonation.

Experimental Section

Preparation of Deuteropyridines. Except for commercially available pyridine-d_5, each deuteropyridine or dideuteropyridine was prepared from the corresponding bromopyridine or dibromopyridine with two portions of excess Zn dust in D_2SO_4/D_2O at 90 °C.[14] 2,6-Lutidine-2,6-$(CD_3)_2$ was prepared by two successive exchanges in D_2O containing K_2CO_3 at 180 °C,[15] but in a Biotage Initiator microwave oven at 15 bar. Incorporation of deuterium to >95% was verified by ^1H NMR.

Preparation and Titration of NMR Samples. Pyridine samples (~1:1 H/D) were prepared as 1.0 M solutions in D_2O with 1,4-dimethoxybenzene as the internal standard (δ 114.5) and with a small aliquot of $NaOD/D_2O$ to guarantee that the pyridine was fully deprotonated. The 2,6-lutidine sample was prepared similarly, but in 20% DMSO-d_6 in D_2O, owing to a lower solubility. The samples were then degassed under vacuum. Successive 5-μL aliquots of 4.0 M DCl/D_2O were added and ^{13}C NMR spectra were recorded after each addition, until there was no further change in chemical shifts. The number of aliquots varied from 12 to 20. For each deuterated pyridine isotopologue at least two carbons undergo substantial changes in chemical shifts upon *N*-protonation and were thus suitable as reporter nuclei. When necessary, signals of the isotopologues were assigned from the stoichiometry or by adding authentic undeuterated material. For carbons attached to D the chemical shift of the central component of the CD triplet or of the CD_3 septet was used.

Instrumentation. All ^1H NMR spectra and all ^{13}C NMR spectra of lutidine were recorded on a JEOL ECA500 spectrometer. All ^{13}C NMR spectra of pyridines were recorded on a Varian VX-500 spectrometer (125 MHz), using an XSense direct-detection cold probe. A 7.0-μs, 45° excitation pulse, 128−800 acquisitions, 32K data points zero-filled to 128 K, and standard broad-band ^1H decoupling were used, with the temperature regulated at 298 K.

Data Analysis. Chemical shifts at various pyridine positions during the course of a titration of a pair of isotopologues were analyzed according to eq 1. The resulting values of $K_a{}^H/K_a{}^D$ were converted to ΔpK ($= \log_{10}(K_a{}^H/K_a{}^D)$) and averaged for each isotopologue. Because precision varied with the reporter nucleus, weighted averages were calculated (weighted inversely to the square of the standard deviation), and associated errors are either errors of the weighted average or standard errors of the mean, whichever is larger.

Computations. Ab initio density-functional calculations on pyridine, pyridinium ion, and their 2,6-dimethyl derivatives were performed at the B3LYP/cc-pVTZ level, as recommended,[16] using Gaussian 03, Revision D.01.[17] Harmonic vibrational frequencies for d_0, 2,6-d_2, 3,5-d_2, 4-d, d_5, $(CD_3)_2$, and anti-$(CH_2D)_2$ isotopologues were calculated at very tightly optimized geometries on an ultrafine optimization grid. The contributions from molecular masses and moments of inertia, or from the product of vibrational frequencies via the Redlich−Teller product rule,[18] are negligible and are ignored. The double difference, $\Delta\Delta\Sigma\nu$, of sums of all the frequencies of the four species was calculated according to eq 2. This difference was then converted through zero-point energies to a calculated ΔpK at 298 K. A separate $\Delta\Delta\Sigma\nu$ for high-frequency C−H vs C−D stretching modes (frequencies > 3000 cm^{-1} or 2100 cm^{-1}, respectively) could also be obtained. For the pyridine isotopologues vibrations can be distinguished as in-plane and out-of-plane, according to the *z* components of the displacements, and a $\Delta\Delta\Sigma\nu$ due to all the in-plane vibrations was extracted.

$$\Delta\Delta\Sigma\upsilon = (\Sigma\upsilon_{HPy^+} - \Sigma\upsilon_{HPyd_n{}^+}) - (\Sigma\upsilon_{Py} - \Sigma\upsilon_{Pyd_n}) \quad (2)$$

Results

The intrinsic isotope shift due to deuteration is always to lower frequency (upfield), except for C3,5 of lutidine-2,6-$(CD_3)_2$. ^{13}C NMR chemical shifts of pyridines and pyridine isotopologues at the beginning and end of titration are listed in Table S1. Experimental data from NMR titrations of deuterated pyridine isotopologues are listed in Table 1. For every titration the correlation coefficient *R* for the linear fit to eq 1 was greater than 0.99995, except for C4 of 2,6-lutidine, where *R* drops to 0.9998 because the intrinsic isotope shift of the distant deuteriums is insufficient to resolve the isotopologues near the beginning and end of the titration. Figure 1 shows not a typical plot, but the *second worst* such plot, for C2,6 of pyridine-d_5, for which the slope is 1.0828 ± 0.0030 but for which *R* is only 0.99995.

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Table 1. Deuterium IEs on Basicity As Measured at Each Reporter Nucleus, Average IEs, and Average IE per D

Py-d_n	^{13}C	K_a^H/K_a^D	average ΔpK	ΔpK per D
2,6-D_2	C2,6	1.0139 ± 0.0016	0.0060 ± 0.0002	0.0030 ± 0.0001
2,6-D_2	C3,5	1.0141 ± 0.0005		
3-D	C2,6	1.0258 ± 0.0010	0.0104 ± 0.0006	0.0104 ± 0.0006
3-D	C4	1.0229 ± 0.0002		
3,5-D_2	C2,6	1.0523 ± 0.0010	0.0212 ± 0.0004	0.0106 ± 0.0002
3,5-D_2	C3,5	1.0493 ± 0.0006		
3,5-D_2	C4	1.0487 ± 0.0002		
4-D	C3,5	1.0174 ± 0.0004	0.0074 ± 0.0001	0.0074 ± 0.0001
4-D	C4	1.0172 ± 0.0002		
D_5	C2,6	1.0828 ± 0.0030	0.0343 ± 0.0006	—
D_5	C3,5	1.0825 ± 0.0023		
D_5	C4	1.0809 ± 0.0025		
2,6-$(CD_3)_2$	C2,6	1.0703 ± 0.0005	0.0294 ± 0.0010	0.0049 ± 0.0002
2,6-$(CD_3)_2$	C3,5	1.0699 ± 0.0007		
2,6-$(CD_3)_2$	C4	1.0660 ± 0.0063		
2,6-$(CD_3)_2$	CH_3	1.0692 ± 0.0015		

Table 2. Calculated Secondary Isotope Effects on Basicities of Deuterated Pyridines

Py-d_n	n^a	$\Sigma\nu_{Pyd_n}$	$\Sigma\nu_{HPyd_n}+$	$\Delta\Delta\Sigma\nu$, cm^{-1}	ΔpK	$\Delta\Delta\Sigma\nu$, cm^{-1b}	$\Delta\Delta\Sigma\nu$, cm^{-1c}
Py	—	38 900	45 168	0	0	0	0
2,6-d_2	2	35 998	42 243	22.8	0.024	28.4	26.2
3,5-d_2	3	36 043	42 261	49.4	0.052	13.9	31.5
4-d	4	37 467	43 717	18.1	0.019	5.1	12.6
d_5	2, 3, 4	31 704	37 881	90.3	0.095	47.3	70.5
2,6-$(CH_3)_2$	—	62 719	68 951	0	0	0	—
2,6-$(CD_3)_2$	3	54 367	60 562	37.2	0.039	17.0	—
2,6-$(CH_2D)_2$[d]	3	59 926	66 137	21.0	0.022	19.0	—

[a] Number of bonds between D and N. [b] C−H/D stretches. [c] In-plane vibrations. [d] Anti D.

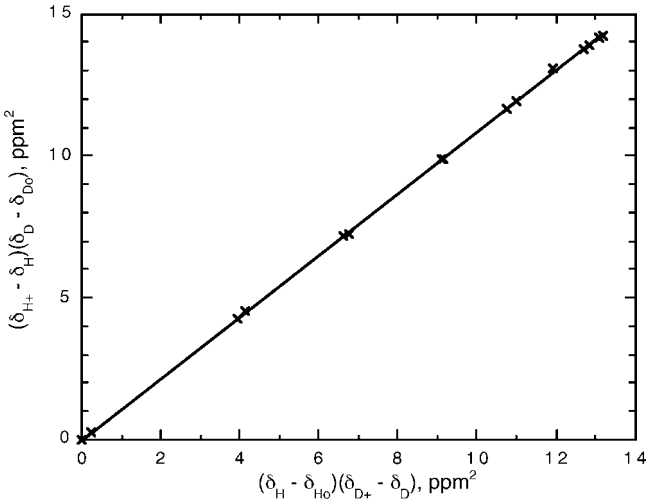


Figure 1. Linearized plot (eq 1) for C2,6 signals in NMR titration of pyridine plus pyridine-d_5.

The data in Table S1 verify that the chemical shifts of C3 and C4 move to higher frequency (downfield) on *N*-protonation, whereas those of C2 and 2-CH_3 move to lower frequency, despite the depletion of electron density. This reversal does not spoil the applicability of eq 1. However, the sensitivity of chemical shift to the state of protonation is greatest for C4 and least for C3,5. Consequently the precision of the IE generally increases from C3,5 to C2,6 to C4, except when the reporter nucleus was a deuterated carbon, for which the signal was weak.

The data are internally consistent. The IE measured for each isotopologue is the same, within a small but variable experimental error, regardless of reporter nucleus. The IE of pyridine-3,5-d_2 is double that of pyridine-3-d, or equivalently, the IE per D is the same. The sum of the ΔpK values of pyridine-2,6-d_2, -3,5-d_2, and -4-d is 0.0347 ± 0.0005, which agrees very well with 0.0343 ± 0.0006 of pyridine-d_5. Both of these comparisons show that the IEs at the various positions are additive.

Computational results on deuterated pyridine isotopologues are listed in Table 2. The values for the total zero-point energies for pyridine, pyridinium ion, and their d_5 isotopologues differ from the ones calculated previously[11] by no more than 4 cm^{-1}. A separate $\Delta\Delta\Sigma\nu$ for high-frequency C−H vs C−D stretching modes is also included, as well as $\Delta\Delta\Sigma\nu$ from in-plane vibrations for pyridine isotopologues.

Discussion

The data show that deuteration at any position increases the basicity of pyridines. This is consistent with the general phenomenon seen in many previous studies of secondary deuterium IEs on acidity and basicity.

According to Table 1, the ΔpK_a value for pyridine-d_5 is 0.0343 ± 0.0006. This result settles the disagreement between the two previously measured values of 0.02 and 0.11.[9,10] Both are wrong, but the former one is closer to the correct value, probably fortuitously, because both measurements were subject to severe interference from vibrational fine structure in the UV spectra. Certainly our result is much more reliable, inasmuch as even the worst entries in Table 1 are more precise than the ±0.02 ΔpK_a of those previous studies.

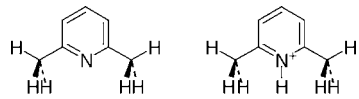
Furthermore, the data in Table 1 show that we can separate the contributions from each of the positions around the pyridine ring. For the isotopologues of pyridine the IE per deuterium is largest for pyridine-3,5-d_2 and smallest for pyridine-2,6-d_2, even though in the latter the deuterium substitution is closest to the site of *N*-protonation. The IE per deuterium in 2,6-lutidine is also small, even though the methyl groups are again quite close to the site of *N*-protonation. It is larger though than the IE in

pyridine-2,6-d_2. These are unexpected results, and the observation that the largest IE is from deuteration at C3,5 is quite surprising.

The calculations in Table 2 corroborate the experimental observations that deuteration at any position increases the basicity of pyridines. Because the calculated IEs are based on changes in vibrational frequencies on *N*-protonation, the correspondence between calculated and experimental implies that the IEs arise from zero-point energies. This excludes the possibility that the major contributor to the IEs is from inductive effects arising from anharmonicity, which makes a C−H bond longer than a C−D bond and changes the dipole moment.[19] Such an origin was suggested for pyridine and some substituted pyridines,[9] and it cannot be rejected as a minor contributor, but it is not the dominant contributor. This is the same conclusion that was reached for secondary deuterium IEs on the basicity of amines, where the IE could be shown to be of stereoelectronic origin, reaching a maximum when the deuterium is antiperiplanar to the nitrogen lone pair.[7]

The values in Table 2 separate the contributions from each of the positions around the pyridine ring. The calculated IEs, per deuterium, decrease in the order pyridine-3,5-d_2 > pyridine-4-d > lutidine-2,6-$(CD_3)_2$ > pyridine-2,6-d_2, which is the same as the order obtained experimentally, except for a reversal of the two lowest. Therefore we can be confident that the IE does not fall off with distance from the site of *N*-protonation, but that the smallest IEs are seen at the positions nearest the nitrogen.

In both 2,6-lutidine and 2,6-lutidinium ion the lowest-energy conformation of each methyl is calculated to have one hydrogen antiperiplanar to the nitrogen and two hydrogens syn (**1a**,**1b**). The calculated ΔpK due to deuteriums in those two anti positions is nearly half of that due to six deuteriums. Therefore the anti deuteriums contribute a larger IE, per deuterium, than do syn deuteriums. Of course, this feature of the calculation could not be verified experimentally.



1a **1b**

Comparison of Tables 1 and 2 shows that the calculated IEs are larger than the experimental ones, by an average factor of 2.5. This overestimate arises because hydrogen bonding by solvent to the nitrogen lone pair makes the pyridine resemble the pyridinium ion, thereby reducing the magnitude of the IE. A similar discrepancy was obtained for acidities of carboxylic acids and phenols.[8]

Computations that take solvation into account would be more realistic. Rather than attempt them on pyridines, we carried out B3LYP/6-31 calculations on formic acid, both without solvation (as previously)[8] and with the polarized continuum model of water. According to the vibrational frequencies in Table S8, the double difference, $\Delta\Delta\Sigma\nu$, of sums of all the frequencies (eq 2) and thus the calculated secondary deuterium IE on the acidity of formic acid are reduced by 50% in this solvent. This is an imperfect model, in that continuum solvation ignores the specific hydrogen-bonding feature of water, but it does support a strong role for solvation to reduce the IE.

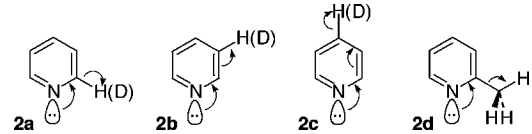
A higher gas-phase IE, corresponding to a ΔpK_a of 0.09, as observed and also calculated for pyridine-d_5,[11] further supports a substantial role for solvation in reducing the IE. That IE coincides with the 0.11 obtained earlier in aqueous solution,[10] but the agreement must be fortuitous because that aqueous value is wrong.

It is difficult to assign the IEs to specific isotope-sensitive vibrations. Because of variations in coupling of the individual bond vibrations into normal modes, a frequency in pyridine cannot be correlated with one in pyridine-d_n or in a pyridinium ion. Prime candidates though are the C−H stretching modes, whose vibrational frequencies and zero-point energies increase on *N*-protonation. According to the calculations in Table 2, stretching modes are indeed largely responsible for the small IE in pyridine-2,6-d_2 and for the IE of the anti D in lutidine-2,6-$(CH_2D)_2$, but not for the other, larger IEs.

Another separation of vibrational modes in pyridine is between in-plane and out-of-plane. According to the results in Table 2, the in-plane modes contribute nearly 80% of the IE in pyridine-d_5 and all of the small IE in pyridine-2,6-d_2. This is consistent with the previous conclusion regarding stretching modes of pyridine-2,6-d_2, which are necessarily in-plane. Less than 20% of the overall IE in pyridine-d_5 comes from the out-of-plane bending modes, which represent 30% of the total modes.

Alternatively, the IE in pyridine-d_5 has been associated with a calculated shortening of the C−H distance and a strengthening of the C−H bond on *N*-protonation.[11] However, the calculated shortening decreases in the order C2−H > C3−H > C4−H, whereas the IEs do not vary monotonically with distance. Instead the largest IE is in pyridine-3,5-d_2. Likewise, the redistribution of electron density does not account for the IEs, inasmuch as H2,6 are the hydrogens calculated to acquire the greatest positive charge on *N*-protonation, and they show the smallest IE.

It is tempting to try to assign the IEs to n-σ^* delocalization, which weakens C−H bonds and lowers zero-point energies, as can be recognized in amines and the formate anion.[7,8] Delocalization of the nitrogen lone pair into σ^*_{CH}, as in **2a** and **2c**, would lower the stretching frequency and zero-point energy of the C2−H and C4−H bonds in the neutral pyridine. This delocalization would lead to a greater IE for deuteration at C2 and C4, where the observed IEs are smaller. In contrast, for the C3−H and anti-2-CH_3 bonds the lone pair is delocalized instead into σ_{CH}, as in **2b** and **2d**. This delocalization would increase the stretching frequency and zero-point energy of these bonds in the neutral pyridine and would lead to an inverse IE, which is not seen. This may account though for why the IE of the 2-CH_3 is so small. It does not account for the surprising observation that pyridine-3,5-d_2 shows the largest IE, little of which arises from stretching modes that would be strengthened by delocalization as in **2b**. Therefore it seems futile to associate the IEs with specific features of bonding, structure, or vibrational modes.



2a **2b** **2c** **2d**

Conclusions

We have used an NMR titration method to accurately measure secondary IEs on the basicities in aqueous solution

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of various deuterated pyridine isotopologues and of 2,6-lutidine-2,6-$(CD_3)_2$. The data show that deuteration at any position increases the basicity. The IE per deuterium is largest for substitution at the 3-position and smallest for the 2-position, which is closest to the site of *N*-protonation, smaller even than that for 2-CD_3 substitution. Computations can reproduce the experimental trends with isotopic position, although they overestimate the magnitude of the measured IEs, owing to the neglect of solvation. According to the calculations, the IEs originate from vibrations whose frequencies and zero-point energies are increased on *N*-protonation, but it is not possible to associate those increases with individual vibrational modes or with n-σ* delocalization. It is also not possible to account for the surprising observations, confirmed by calculation, that the IE is smallest for deuteration at the nearby C2 or 2-methyl.

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Supporting Information Available: Complete ref 17. Table S1 (^{13}C chemical shifts of pyridines and pyridines-d_n at beginning and end of titration). Tables S2−S5 (calculated energies, optimized geometries, atomic charges, and interatomic distances of all structures). Tables S6−S7 (calculated vibrational frequencies for pyridine, pyridinium ion, 2,6-lutidine, and 2,6-lutidinium ion and their deuterated isotopologues). Table S8 (calculated vibrational frequencies for formic acid, formate, and their deuterated isotopologues, without and with a polarized continuum model of solvation). This material is available free of charge via the Internet at http://pubs.acs.org.

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