Nitrogen Kinetic Isotope Effects on the Decarboxylation of 4-Pyridylacetic Acid

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Nitrogen kinetic isotope effects on the decarboxylation of 4-pyridylacetic acid have been measured in solvents of different polarity and have been found to vary from the inverse value of 0.994 to the normal value of 1.002 upon increase of water content of the binary dioxane–water solvent from 25% to 75% (v/v), respectively. These changes were successfully modeled theoretically and shown to originate from the large inverse nitrogen isotope effect on the equilibrium between acidic and zwitterionic forms.

Introduction

Because of the importance of the carboxylation/decarboxylation reactions in the carbon cycle, decarboxylation reactions have been studied extensively in enzymatic systems[1] and their chemical models.[2] Their mechanism sometimes provokes intensive debate, as, for example, the mechanism of the orotidine monophosphate decarboxylase.[3] However, theoretical modeling of decarboxylation reactions[4] in general, and those involving zwitterions in particular, is scarce. This is because zwitterionic species are not reliably modeled theoretically since they are unstable in the gas phase. Modeling of these processes was, therefore, retarded until suitable methods of solvent treatment became available.

We have recently succeeded[5] in modeling the decarboxylation of 4-pyridylacetic acid **A** and validated our
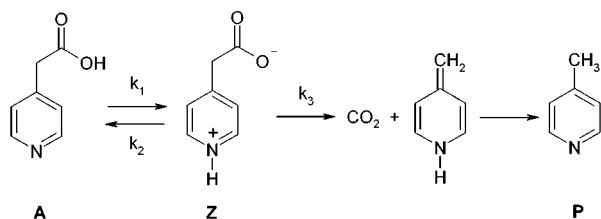
theoretical findings by comparing calculated kinetic isotope effects (KIEs) of carboxylic carbon and oxygen to

the experimental values obtained by O'Leary and co-workers.[6,7] This decarboxylation proceeds through the zwitterion **Z** to form carbon dioxide and 4-methylpyridine **P**.

We have found the transition-state structure of this reaction to be extremely sensitive to the properties of the solvent. It is of great importance to further verify this theoretical finding by experimental results. The dependence of the structure of the transition state on the solvent polarity can help to shed some light on the mechanism of decarboxylation reactions catalyzed by the PLP-dependent enzymes. For these enzymes, neutralization of the charges of the zwitterion placed in a nonpolar active site is considered to be the driving force of the rate enhancement. These reasons prompted us to extend the amount of the experimental data, which can be used for fine-tuning of calculations, and provide additional insight into the nature of the spontaneous and enzyme-catalyzed decarboxylation reactions.

We report here determination of the nitrogen KIEs on the decarboxylation of 4-pyridylacetic acid in 25%, 50%, and 75% (v/v) binary dioxane–water mixtures. We have found a systematic increase of the nitrogen KIE with the increase of water content, in very good agreement with an increasing trend predicted theoretically.

Results and Discussion

Experimental studies of the ^{15}N KIEs on the 4-pyridylacetic acid were performed in the binary 1,4-dioxane–water solvents of three compositions, 25%, 50%, and 75% v/v, i.e., compositions for which carboxylic carbon and oxygen KIEs have been detrermined.[6,7] The range is limited by the reaction being too slow in pure water and too fast in pure dioxane to allow determination of the isotope effects. However, the relative first-order rate constants are 1:30:211 for 25%, 50%, and 75% v/v water contents, respectively, providing the range sufficient for studies of solvent polarity on KIEs. The product was

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(1) For a review see: O'Leary, M. H. *Acc. Chem. Res.* **1988**, *21*, 450.
(2) Willi, A. In *Carbon-13 in Organic Chemistry*; Buncel, E., Lee, C. C., Eds; Isotopes in Organic Chemistry, Vol. 3; Elsevier: Amsterdam, 1977; p 237.
(3) (a) Lee, J. K.; Houk, K. N. *Science* **1997**, *276*, 942. (b) Warshel, A.; Štajbl, M.; Villà, J.; Florián, J. *Biochemistry* **2000**, *39*, 14728. (c) Wu, N.; Mo, Y.; Gao, J.; Pai, E. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2017. (d) Houk, K. N.; Lee, J. K.; Tantillo, D. J.; Bahmanyar, S.; Hietbrink, B. N. *ChemBioChem* **2001**, *2*, 113. (d) Rishavy, M. A.; Cleland, W. W. *Biochemistry* **2000**, *39*, 4569.
(4) (a) Bach, R. D.; Canepa, C. *J. Am. Chem. Soc.* **1997**, *119*, 11725. (b) Cordeiro, M. N. D. S.; Dias, A. A.; Costa, M. L.; Gomes, J. A. N. F. *J. Phys. Chem. A* **2001**, *105*, 3140. (c) Czyryca, P.; Paneth, P. *J. Org. Chem.* **1997**, *62*, 7305.
(5) Sicinska, D.; Truhlar, D. G.; Paneth, P. *J. Am. Chem. Soc.* **2001**, *123*, in press.

(6) Marlier, J. F.; O'Leary M. H. *J. Am. Chem. Soc.,* **1986**, *108*, 4896.
(7) Headley, G. W.; O'Leary, M. H. *J. Am. Chem. Soc.* **1990**, *112*, 1894.

Table 1. Comparison of the Experimental and Theoretical Nitrogen KIEs on the Decarboxylation of 4-Pyridylacetic Acid at Room Temperature

	dioxane SM5.42	25% water	50% water	75% water	water		
					SM5.42	COSMO	3 explicit water molecules
theory	0.9825[a]				1.0082	1.0079	1.0066
experiment		0.994 ± 0.002^{b}	0.997 ± 0.001	1.0019 ± 0.0004			

[a] All calculations at HF/6-31G(d) level. [b] Standard deviation is reported.

isolated from the reaction mixture and subjected to isotope-ratio mass spectrometric analysis. Natural isotopic composition was used; thus, no isotopic synthesis was necessary. KIEs were calculated from the equation

$$k_{14}/k_{15} = \frac{\ln(1 - f)}{\ln[1 - f(1000 + \delta_f)/(1000 + \delta_\infty)]} \quad (1)$$

where f is the fraction of reaction and δ values are isotopic ratios, relative to a standard, obtained from the mass-spectrometric measurements of the product after full conversion (δ_∞) and after the fraction of reaction f (δ_f). Air nitrogen was used as the local standard; its isotopic composition does not affect the isotope effect (cf. eq 1). For reactions carried out in solvents containing 25% and 50% water, duplicate independent measurements were made at the fraction of reaction of about 0.1. The smaller the fraction of reaction the larger the difference between δ_f and δ_∞ and, thus, the more reliable the determination of KIEs. For the reaction carried out in the solvent containing 75% of water more thorough studies were performed. These included nine independent measurements with the fraction of reaction ranging from 0.05 to 0.30. Obtained values for all three solvents are listed in Table 1. Nitrogen KIEs show increasing trend; at low water content the nitrogen KIE is inverse (smaller than unity, indicating that the heavier isotope is reacting faster than the light one). At 50% dioxane−water mixture the nitrogen KIE is still inverse but closer to unity and becomes normal at water rich composition. These values are within the range predicted theoretically by computing nitrogen KIEs for pure dioxane and the aqueous solution. Also the observed trend in the change of the nitrogen KIE agrees with the theoretical prediction. Analysis of the calculations performed with the SM5.42 continuum solvent model[8] of dioxane and aqueous solutions indicates that the mechanism changes with the change of the solvent polarity.[9] In aqueous solution, the zwitterionic form is more stable than the acid and thus the reaction proceeds from the zwitterion **Z** to the transition state **T**. In pure dioxane, however, the zwitterion is less stable than the acid. Thus, the reaction can be viewed as a stepwise process and the nitrogen KIE can be calculated as a product of the isotope effect on the equilibrium between acid **A** and zwitterion **Z** (EIE) and KIE on the conversion of the zwitterion to product:[10]

$$\text{KIE} = (\text{KIE}_1/\text{KIE}_{-1})\text{KIE}_2 = \text{EIE} \cdot \text{KIE}_2 \quad (2)$$

In Table 1, we have reported values calculated theoretically at the SM5.42/HF/6-31G(d) level for pure dioxane and for aqueous solution. We did not attempt

Table 2. Properties of the NH Moiety Calculated on at the SM5.42/HF/6-31G(d) Level

	dioxane			water		
	A	**Z**	**T**	**A**	**Z**	**T**
$d(\text{N})^a$	−0.37	−0.26	−0.33	−0.37	−0.26	−0.34
$d(\text{H})$		0.37	0.35		0.37	0.35
$r(\text{N−H})$		0.998	1.003		1.009	1.002
$n(\text{N−H})^c$		0.74	0.76		0.74	0.77
$n(\text{N−C})$	1.40	1.13	1.06	1.40	1.13	1.05

[a] CM2 atomic partial charges.[14] [b] Bond lengths in Å. [c] Mayer bond order.[15]

calculations for the mixed solvents because current solvation models are not ready to address problems connected with the preferential solvation, which has been postulated for dioxane−water mixtures.[11] Interestingly, this nitrogen KIE in water is modeled equally well by the COSMO solvent continuum model,[12] as well as a model that contains three explicit water molecules: two in the vicinity of the carboxyl group and one close to nitrogen atom. This is not the case for the oxygen EIE on the same reaction, which seems to indicate that modeling of nitrogen isotope effects is less demanding than modeling of oxygen isotope effects. The value obtained for the reaction in dioxane can be further split into its components. We have calculated EIE = 0.9756 and KIE$_2$ = 1.0071. As can be seen, it is the equilibrium isotope effect that makes the overall KIE so different in dioxane and in water. The EIE calculated here is in excellent agreement with the experimental values of 0.978, 0.979, and 0.981 recalculated from the nitrogen EIEs on the deprotonation of 3-acetyl-, 4-methyl-, and pyridine, respectively.[13]

The comparison of the main properties of the nitrogen atom and the hydrogen atom bonded to it for all the reacting species in both solvents is given in Table 2. As can be seen, all calculated properties are very similar in both solvents, the only exception being the length of the N−H bond, which is stretched in aqueous solution due to hydrogen bonding.

Experimental Section

Materials. 4-Pyridylacetic acid hydrochloride (98%) was obtained from Aldrich. Chloroform (pure p.a.), 1,4-dioxane

(8) (a) Li, J.; Hawkins, G. D.; Cramer, C. J.; Truhlar, D. G. *Chem. Phys. Lett.* **1998**, *288*, 293. (b) Li, J.; Zhu, T.; Hawkins, G. D.; Winget, P.; Liotard, D. A.; Cramer, C. J.; Truhlar, D. G. *Theor. Chem. Acc.* **1999**, *103*, 9. (c) Zhu, T.; Li, J.; Liotard, D. A.; Cramer, C. J. *J. Chem. Phys.* **1999**, *110*, 5503.

(9) Actually, the SM5.42 model includes several factors connected with the solvation, including cavitation energy and hydrogen bonding. The detailed influence of these factors on the rate of the decarboxylation of 4-pyridylacetic acid will be discussed elsewhere. We make a point here that of all factors, electrostatic effects play the dominant role.

(10) Note that, from the thermodynamic point of view, the overall isotope effect can be calculated directly from the reaction of **A** going to transition state **T**. Analysis of the isotopic fractionation assuming intervention of the zwitterion, and using eq 2, is, however, more chemically intuitive and informative.

(11) (a) Kinart, W. J.; Kinart, C. M.; Skulski, L. *Pol. J. Chem.* **1989**, *63*, 581. (b) Skulski, L.; Kinart, C. M. *Pol. J. Chem.* **1992**, *66*, 287.

(12) Klamt, A.; Schüürmann, G. *J. Chem. Soc., Perkin Trans. 2* **1993**, 799.

(13) Kurz, J. L.; Pantano, J. E.; Wright, D. R.; Nasr, M. M. *J. Phys. Chem.* **1986**, *90*, 5360.

(spectrophotometric grade), KOH (pure p.a.), and HCl (pure p.a.) were supplied by P.O.Ch. Gliwice, Poland. These were used without further purification. Water was purified by the Nanopure II system (Barnstead).

Equipment. The absorption spectra were recorded using a Cary 5E (Varian) spectrophotometer in a 1 cm quartz cell. The resolution was 0.1 nm. The pH settings were controlled with the aid of pH/Ion Analyzer MA235 Mettler Toledo. Standard ^{1}H NMR spectra were obtained on a Bruker DPX250. Isotope ratios were measured on a Europa 20-20 isotope-ratio mass spectrometer.

Kinetics. 4-Pyridylacetic acid was dissolved in water, the pH of the solution was brought to 4.0 with KOH, and the solution was thermostated at room temperature. The reaction was initiated by addition of the cosolvent, 1,4-dioxane, to yield the final mixed-solvent composition of 25, 50, 75% (v/v). At intervals, aliquots of reaction mixture were withdrawn, and the reaction progress was estimated using ^{1}H NMR analysis in DMSO. At the desired fractions of reaction, the exact fraction of reaction was determined in the spectrophotometric assay as described by O'Leary and Marlier.[6] Namely, 20 μL of the solution was withdrawn and injected into 3.0 mL of water with 0.1 mL of 1.0 M KOH. Then the product was extracted with 4 mL of CHCl$_3$, and the reactant concentration was determined by measuring optical density at 257 nm of the aqueous layer (molar extinction coefficient of 4-pyridylacetic acid equals to 2590 M^{-1} cm^{-1} at 25 °C). Simultaneously, the bulk of the sample (approximately 25 mL) was brought to pH = 10−11 with KOH and shaken vigorously with a 10-fold excess of CHCl$_3$. Under these conditions, the product, 4-methylpyridine, was quantitatively extracted into the organic layer, while the reactant was retained in the aqueous phase. After separation, the organic phase was acidified with the concentrated HCl in order to convert amine to the nonvolatile salt and placed in an evaporator, and the solvent was removed under reduced pressure. Because the product is highly hygroscopic, samples were placed in a vacuum desiccator, first over molecular sieves and then over P$_2$O$_5$, for final drying and storage. 4-Methylpyridine was then used for the isotopic analysis of nitrogen.

Isotopic Analysis. Samples of about 2 mg of 4-methylpyridine per measurement were used. Isotope ratio measurements were performed using a Europa Scientific 20-20 analyzer with an ANCA-SL preparation module for the on-line combustion of bulk solid samples. The natural isotopic composition of nitrogen was used.

Calculations. As described in details elsewhere,[5] all calculations involving the SM5.42 solvation model[10] were performed using the Gaussian98[16] and the MN-GSM[17] programs.

MC-QCISD energy calculations on the SM5.42/HF/6-31G(d)-optimized geometries of **A**, **Z**, and **T** in dioxane and in water were performed using the MULTILEVEL program.[18] Vibrational analysis was performed for each stationary point, minima and transition states were confirmed to have zero and one imaginary frequency, respectively. Hessians were used for calculations of KIEs according to the complete equation of Bigeleisen[19] by means of the Isoeff98 program.[20]

Conclusions

We have shown that nitrogen KIE changes from the inverse value of 0.994 to the normal value of 1.002 upon increase of water content of the binary dioxane−water solvent from 25% to 75% (v/v), respectively. This change was predicted theoretically and shown to originate from the large inverse nitrogen EIE on the equilibrium between acidic and zwitterionic forms.

(14) Li, J.; J.; Zhu, T.; Cramer, C. J.; Truhlar, D. *J. Phys. Chem. A* **1998**, *102*, 1820.

(15) Mayer, I. *Chem. Phys. Lett.* **1983**, *97*, 270.

(16) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T. A.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Peng, C. Y.; Ayala, P. A.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 98*; Gaussian, Inc.: Pittsburgh, PA, 1998.

(17) Xidos, J. D.; Li, J.; Hawkins, G. D.; Liotard, D. A.; Zhu, T.; Rinaldi, D.; Cramer, C. J.; Truhlar, D. G.; Frisch, M. J. *Minnesota Gaussian Solvation Module MN-GSM-version 99.6*; University of Minnesota: Minneapolis, 2001.

(18) Rodgers, J. M.; Lynch, B. J.; Fast, P. L.; Chuang, Y.-Y.; Pu, J.; Truhlar, D. G. *MULTILEVEL-version 2.2*; University of Minnesota: Minneapolis, 2001.

(19) Melander L.; Saunders, W. H., Jr. *Reaction Rates of Isotopic Molecules*; Wiley and Sons: New York, 1980.

(20) Anisimov, V.; Paneth. P. *J. Math. Chem.* **1999**, *26*, 75.