energies are very incompletely expressed. On the other hand, the critical complexes still have considerable charge separation. The residual negative charge of the BH_4^- unit is much closer to the positive charge on N in the critical complex for 1,2-addition than in that for 1,4-addition.

The value of α_{-1}, for the hydroxylation of the quinolinium ions, is also in reasonable accord with the Marcus theory. W^r and W^p were both assumed to be zero, and τ was taken as 1. Then, using central values of $\Delta G°$ and ΔG^* ($\Delta G° = -50.6$ kJ mol^{-1}; $\Delta G^* = 36.8$ kJ mol^{-1}) eq 6[46] gave a value of 238 kJ mol^{-1} for λ. This is a very satisfactory outcome, because it is a little over half of the well-determined λ_i for hydride transfer between quinolinium ions, 389 kJ mol^{-1}.[6] The quinolinium reactant in that reaction is undergoing changes very analogous to those in the present reaction. A covalent bond is being formed at C-4, and the positive charge is being neutralized. However, the "donor", instead of being a dihydroquinoline, which would match the contributions of the acceptor to λ, is simply OH^-, which probably contributes only a little desolvation energy to λ. Thus λ for hydroxide addition to quinolinium ions is expected to be a little over half of 389 kJ mol^{-1}.

Using this value of λ and the other parameters already selected, eq 12 gives 0.39 for α_{-1}. This is in qualitative agreement with the experimental value (0.23) in that both are substantially less than 0.5 and indicates a critical configuration closer to reactants that to products. There is a substantial quantitative gap between them, but the uncertainty in α may account for a good deal of it. It may be significant that a similar treatment of an α value for a similar reaction in aqueous solution *underestimates* α.[10]

As we have already concluded for the reaction with BH_4^-, the Marcus theory can be applied reactions which depart widely from the model for which it was derived. The qualitative conclusions which are reached seem reasonable. In particular, the idea that the intrinsic barrier can be approximated by adding independent contributions from each of the reactants, embodied in eq 10, seems to be strongly supported. This was anticipated by Hine[19] some time ago. However, too much physical significance should not be given to the numerical values of the parameters, as these values may well be compensating for the inadequacies of the model. These conclusions are probably applicable to proton transfer as well.[47]

(46) Equation 6 was rearranged to give an explicit solution for λ; $\lambda = 2[\Delta G^* - \Delta G°'/2 + (\Delta G^{*2} - \Delta G^* \Delta G°')^{1/2}]$.

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Solvent Dependence of Oxygen Isotope Effects on the Decarboxylation of 4-Pyridylacetic Acid

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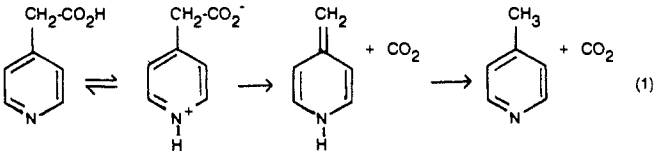
Abstract: Oxygen isotope effects on the decarboxylation of 4-pyridylacetic acid have been measured by the remote-label technique. The isotope effect varies from $k^{16}/k^{18} = 0.995$ per oxygen in 25% dioxane to 1.003 in 75% dioxane. The isotope effect reflects three contributions: An inverse isotope effect of 0.98–0.99 due to the change in carbon–oxygen bond order on going from ground state to transition state, an effect of 1.01–1.02 due to desolvation of the carboxyl group, and an effect of approximately 1.01 due to the acid–base equilibrium of the carboxyl group. Thus, oxygen isotope effects on decarboxylation should be a useful probe for carboxyl desolvation in enzymatic decarboxylations.

In its simplest form, decarboxylation involves the cleavage of a carbon–carbon bond of a carboxylic acid or, more commonly, a carboxylate anion, forming carbon dioxide and leaving behind an organic residue with an unshared pair of electrons. In general, the organic product stabilizes this electron pair, usually by delocalization. Such delocalization is particularly important in enzymatic reactions, where an "electron sink" is generally provided by a coenzyme (e.g., pyridoxal 5'-phosphate, thiamine pyrophosphate), by an enzyme-bound metal ion (various oxidative decarboxylases), or by a prosthetic group (lysine amino group, covalently bound pyruvate).

Rates of decarboxylations are also influenced by the polarity of the medium within which the reaction occurs. In most decarboxylations the transition state is less polar than the ground state (i.e., the negative charge is more delocalized), and consequently decarboxylations proceed more rapidly in less polar solvents. A particularly dramatic example of this effect is the decarboxylation of the pyruvate–thiamine complex studied by Crosby, Stone, and Lienhard,[1] in which the rate increases 9000-fold on going from water to ethanol. Kemp and collaborators have shown that decarboxylation of benzisoxazole-3-carboxylic acids also shows very large medium effects.[2] Rate differences as large as 900 000-fold are observed on going from water to hexamethylphosphoramide. The decarboxylation of 4-pyridylacetic acid (eq 1) also shows large medium effects,[3] with the rate increasing 4000-fold on going from water to 75% dioxane.



(1)

In addition to the general polarity effect, a specific effect of carboxyl group solvation is also important. The starting carboxylate ion, being charged and polar, is extensively solvated. The product of the decarboxylation, CO_2, being nonpolar, is not ex-

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tensively solvated. During the course of the decarboxylation, the carboxyl group solvation is lost. Solvents or systems in which the carboxyl group is less highly solvated result in higher rates of decarboxylation.

Similar solvent effects may be important in enzymatic decarboxylations, although direct evidence is hard to obtain. First, enzymes may provide a nonpolar environment favorable to decarboxylation. Carbon isotope effects on the enzymatic decarboxylation of arginine are solvent dependent and suggest that the rate of the decarboxylation step shows a large medium dependence.[4] Second, binding of the substrate to the enzyme may require desolvation of the substrate carboxyl group; thus, binding energy may be used to do the work of desolvation. Carbon isotope effects on the decarboxylation of homoarginine and norarginine by arginine decarboxylase suggest that carboxyl group desolvation may be associated with binding of the substrate to the enzyme.[4]

Carbon isotope effects have provided important mechanistic information in both enzymatic and nonenzymatic decarboxylations.[5,6] The near constancy of carbon isotope effects in the decarboxylation of 4-pyridylacetic acid, for example, in the face of a 4000-fold change in decarboxylation rate, suggests that although a large change in rate occurs, there is little change in transition state structure accompanying the change in solvent.[7] Carbon isotope effects on enzymatic decarboxylations indicate that in most cases the decarboxylation step is only partially rate-determining.[5]

Although carbon isotope effects can provide important insight into transition-state structures, it is unlikely that carbon isotope effects will be very revealing with regard to the solvation and desolvation of the carboxyl group. Oxygen isotope effects, on the other hand, might be sensitive to such effects. The development of the remote-label technique[8] makes such measurements possible. In this technique a highly labeled substrate containing a $-^{13}C^{18}O_2^-$ group is mixed with isotopically depleted material containing a $-^{12}CO_2^-$ group to form a substrate that contains the natural abundance of ^{13}C, but in which every ^{13}C-containing molecule also contains two ^{18}O. Measurement of the apparent ^{13}C isotope effect actually gives the product of the ^{13}C and ^{18}O effects. Separate measurement of the ^{13}C effect then permits calculation of the ^{18}O effect. This method has previously been used to measure ^{18}O isotope effects on the enzymatic decarboxylation of formic acid.[9]

To provide base line data for studies of oxygen isotope effects on enzymatic decarboxylations, we have measured ^{18}O isotope effects on the decarboxylation of 4-pyridylacetic acid in various solvents. This system shows large medium effects,[3] and we have previously measured carbon isotope effects.[7]

Experimental Section

All solvents were reagent grade. Tetrahydrofuran (THF) (Aldrich Gold Label) was refluxed over sodium benzophenone ketyl and distilled as needed. Anhydrous ammonia (Cardinal Chemical) was condensed from a cylinder as needed. Spectrophotometric grade 1,4-dioxane (Aldrich) was used as supplied. Water was purified with a Millipore Super Q water purification system and had a resistance of at least 18 MΩ. 4-Methylpyridine (Aldrich) was distilled at reduced pressure. 4-Pyridyl acetic acid hydrochloride (Aldrich) was used as supplied. Carbon dioxide (99.9 atom % ^{12}C) and carbon dioxide (99 atom % ^{13}C) were obtained from Isotec. $H_2{}^{18}O$ (97 atom % ^{18}O) was obtained from MSD Isotopes. $^{13}C^{18}O_2$ was prepared by exchanging $^{13}CO_2$ with an excess of $H_2{}^{18}O$.

Equipment. Routine ^1H NMR spectra were obtained on a Bruker WP-200 spectrometer. All chemical shifts are reported in ppm downfield from tetramethylsilane or sodium trimethylsilylpropionate internal reference as appropriate. ^{13}C NMR spectra were obtained on a Bruker AM-500 (125.76 MHz) or a Bruker AM-360 (90.56 MHz) by using sodium trimethylsilylpropionate as an internal reference. UV analyses were performed on a Cary 118.

Isotope ratios were measured on a Finnigan Delta-E isotope ratio mass spectrometer. Isotope ratios have been corrected for the presence of ^{17}O and for instrumental effects.

4-(Sodiomethyl)pyridine. The procedure was modeled after that of Kaiser et al.[10] Liquid NH_3 (1000 mL) was condensed into a 2000-mL, three-necked flask, and freshly cut sodium (62.0 g, 2.70 mol) was added over a 1-h period. Three small pieces of $Fe(NO_3)_2{\cdot}9H_2O$ (5 mm^3) were added to the flask at 1-h intervals. The cooling bath was removed intermittently so that the NH_3 would reflux. When the appearance of the reaction mixture had changed to a light grey precipitate in a colorless liquid (approximately 5 h), the formation of sodium amide was considered to be complete. The flask was then cooled to −78 °C, and 4-methylpyridine (270 mL, 2.77 mols) was added via cannula. THF (700 mL) was then added, the cooling bath was removed, and the flask was purged with N_2 to flush out the NH_3. A further 300 mL of dry THF was added after the solution volume was sufficiently reduced. After the reaction was complete, the solution was transferred via cannula to a glass bottle for storage at −20 °C. The concentration was periodically measured by titrating a small amount of CH_3I and a few crystals of fluorene dissolved in 5 mL of THF with the anion solution. On reaction with D_2O, the anion gave the expected NMR spectrum.

[*carboxyl*-^{12}C]4-Pyridylacetic Acid. An oven-dried, 250-mL, three-necked flask was attached to a vacuum line. The system was evacuated for 15 min, after which the flask and line were filled with dry nitrogen. Approximately 100 mL (0.08 mol) of 4-(sodiomethyl)pyridine solution was then transferred to the flask via a cannula. The flask was gently evacuated to remove any NH_3 and then placed in liquid nitrogen and evacuated. The manifold was filled with $^{12}CO_2$ (600 mL at STP, 0.03 mol), after which the CO_2 was condensed into the flask. The flask was allowed to warm to −78 °C, and the solution was stirred for 12 h, after which it was warmed to room temperature and quenched with 100 mL of water. The solution was then extracted 3× with $CHCl_3$ and 2× with C_6H_6. The aqueous layer was transferred to a 500-mL, round-bottomed flask containing 150 mL of ethanol. The solvents were removed with a rotary evaporator at 40 °C, after which another 150 mL of ethanol was added, and evaporation was repeated. The process of adding ethanol and evaporating was continued until all of the water had been removed. The flask of yellow and white solids was then further dried under vacuum. The solid was acidified with 6 M HCl, which caused the evolution of gas and heat and yielded a bright yellow solution. A 50-mL portion of ethanol was added. Isopropyl alcohol was then added until the precipitation of white inorganic salts had ceased. The mixture was centrifuged at 5000 rpm, 0 °C for 10 min. The supernatant was decanted into a round-bottomed flask, and the solvents were removed with a rotary evaporator. The product was repurified by additional precipitation steps until it was at least 90% pure. The final product was a pale yellow amorphous solid. The final yield was generally about 30% (based on CO_2).

The combined product of five reactions was recrystallized in 20 mL of ethanol. The purity of the combined products was 97% (UV), and the purity of the organic material was 96% (^1H NMR). Thus the overall purity of the material was 93%. The ^{13}C content of the carboxyl group was below the detection limit of ^{13}C NMR, indicating that the percentage of ^{13}C in the carboxyl carbon was less than 0.1%.

[$^{13}C^{18}O_2$]4-Pyridylacetic Acid. The synthesis was carried out as described above, by using $^{13}C^{18}O_2$ on about 1/5 the scale described above.

The ^{18}O content of the compound was determined by the relative intensities of the three ^{13}C carboxyl signals in the NMR. The upfield shift for each ^{18}O was 0.029 ppm. The compound contained 64% $C^{18}O_2$, 28% $C^{18}O^{16}O$, and 8% $C^{16}O_2$.

Carbon Isotope Effects. The labeled and unlabeled 4-pyridylacetic acid were mixed together so that the carboxyl ^{13}C abundance was approximately 1.12%. Isotope effects were measured for this mixed substrate as described previously.[7] For comparison with the previous measurements of Marlier,[7] a series of isotope effect measurements was also carried out on natural-abundance material.

The isotope effect so obtained is approximately equal to the product of the carbon and oxygen isotope effects. The oxygen isotope effect is obtained by dividing the observed isotope effect by the carbon isotope effect[8] and then correcting for the extent of incomplete labeling of the triply labeled material.

Results

The carbon isotope effect on the decarboxylation of 4-pyridylacetic acid in 50% dioxane was measured in order to establish consistency with previous investigations.[7] The isotope effect

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Table I. First-Order Rate Constants, Carbon Isotope Effects, and Oxygen Isotope Effects for the Thermal Decarboxylation of 4-Pyridylacetic Acid in Various Dioxane–Water Mixtures at 25 °C

% dioxane (v/v)	k_{obsd}[a] (s^{-1})	k^{12}/k^{13}[a]	k^{16}/k^{18}[b]
25	$1.8 \pm 0.2 \times 10^{-7}$	1.056 ± 0.0009	0.995 ± 0.0011
50	$5.4 \pm 0.09 \times 10^{-6}$	1.060 ± 0.0011	1.000 ± 0.0011
75	$3.8 \pm 0.08 \times 10^{-5}$	1.064 ± 0.0010	1.003 ± 0.0007

[a] Reference 7. [b] Per oxygen.

obtained, $k^{12}/k^{13} = 1.0606 \pm 0.0019$ for five experiments, was within experimental error of the previous value: 1.0598 ± 0.0011. This confirms the compatibility of the work of the two investigators.

The apparent isotope effect on the decarboxylation of triply labeled [$^{13}C^{18}O_2$]4-pyridylacetic acid was measured by comparing the carbon isotopic compositions after approximately 20% reaction with those after complete reaction. Measurements were made in 25, 50, and 75% dioxane at 25 °C with 0.02 M substrate concentration. Oxygen isotope effects on the decarboxylation were calculated by using these results and the carbon isotope effects of Marlier and O'Leary.[7] The results for five or six measurements in each of the three solvent systems are given in Table I, along with rate constants and carbon isotope effects. Isotope effects are given per oxygen.

Discussion

The decarboxylation of 4-pyridylacetic acid is very medium dependent. As the solvent is changed from 25% dioxane/75% water to 75% dioxane/25% water, the rate increases 200-fold, the carbon isotope effect increases from 1.056 to 1.064, and the oxygen isotope effect (per oxygen) increases from 0.995 to 1.003.

As we have noted previously,[7] the carbon isotope effect is in the range expected for a single-step mechanism in which the carbon–carbon bond cleavage step is totally rate-determining and the carbon–carbon bond is extensively broken at the transition state. The change in carbon isotope effect probably arises from two causes: In the more polar solvent, the substrate exists almost entirely as the zwitterion (eq 1), whereas in the less polar solvent, the substrate is primarily the neutral form, and this change is expected to increase the observed isotope effect by approximately 1.0014, based on previous measurements of the ^{13}C isotope effect on the pK_a of formic acid.[11] The balance of the increase in carbon isotope effect is probably due to a modest change in the extent of carbon–carbon bond breaking at the transition state.

In analyzing the oxygen isotope effect, we assume that the isotope effect arises from changes in bond strength or bond order, rather than from the complete making or breaking of a covalent bond. For this reason, the isotope effect should not have an imaginary frequency factor component but instead only reflects bond order contributions. An increase in bond order will give an inverse contribution to the isotope effect, whereas a decrease in bond order will give a normal contribution.

Several factors contribute to the oxygen isotope effect. First, the increase in carbon–oxygen bond order on going from ground state to transition state should give rise to an inverse oxygen isotope effect. Because carbon isotope effects indicate that the transition-state structure is nearly independent of solvent, this contribution should be nearly solvent-independent. The second factor is solvation. The carboxyl group should be less solvated in the transition state than in the ground state, and this should give rise to a normal oxygen isotope effect. We previously estimated this factor to be 1.01–1.02 for complete desolvation.[9] This is slightly larger than the isotope effect on desolvation of a single water molecule in the conversion of aqueous H_2O to gaseous H_2O, which gives rise to an isotope fractionation of 1.0091.[12] Because of varying substrate solvation with solvent, this contribution might show large variations with solvent. The third factor is interconversion of the zwitterionic form and the neutral form of the substrate (cf. eq 1). In the most polar solvent, the zwitterion predominates, whereas in the least polar solvent, the neutral form predominates.[3] The oxygen isotope effect on the pK_a of formic acid is 1.011 in H_2O,[11] and a similar factor is expected in the present case. Because this isotope effect is normal, the conversion from zwitterion to neutral form will cause the observed oxygen isotope effect to increase as the solvent is made less polar.

In 25% dioxane, the substrate is principally present as the zwitterion; thus, the third contribution is not important. If, as suggested previously,[9] the solvation factor is 1.01–1.02, then the bond order contribution is probably in the range 0.98–0.99.

If the solvent dependence of the oxygen isotope effect is due to changes in ground-state solvation, we would expect that the normal contribution from solvation should decrease as the solvent becomes less polar.[13] Since the bond order effect is solvent independent, this would predict that the oxygen isotope effect should become more inverse as the solvent becomes less polar; this is the opposite of what is observed. Thus, any changes in ground-state solvation must be outweighed by other contributions working in the opposite direction.

On the other hand, the contribution from the zwitterion–neutral (protonation) equilibrium should become larger as the solvent becomes less polar. If all else is constant, the observed isotope effect should increase by approximately 1.01 as the solvent is changed from polar to nonpolar. This is essentially equal to the change observed.

In the enzymatic decarboxylation of formic acid,[9] oxygen isotope effects of 1.005–1.008 were observed, except in the case of one very slow substrate. These isotope effects reflect bond-order contributions and desolvation contributions like those seen here.

In conclusion, three factors contribute to oxygen isotope effects in decarboxylations: The bond-order change, which probably gives rise to an inverse isotope effect of 0.98–0.99, the desolvation effect, which is 1.01–1.02, and the protonation effect, which is near 1.01. The change in oxygen isotope effect in 4-pyridylacetic acid with solvent composition is mostly the result of the protonation effect. Oxygen isotope effects in enzymatic decarboxylations can be used to study the desolvation effect.

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Registry No. $^{18}O_2$, 32767-18-3; ^{13}C, 14762-74-4; $^{13}C^{18}O_2$, 2684-00-6; $C^{18}O_2$, 2537-69-1; $C^{18}O^{16}O$, 18983-82-9; 4-pyridylacetic acid, 28356-58-3; 4-(sodiomethyl)pyridine, 1192-87-6; 4-methylpyridine, 108-89-4; [$^{13}C^{18}O_2$]4-pyridylacetic acid, 124992-39-8; [$^{12}C^{18}O_2$]4-pyridylacetic acid, 124992-40-1; [$^{12}C^{18}O^{16}O$]4-pyridylacetic acid, 124992-41-2.

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(13) The arguments here about changes in ground-state solvation with solvent might also apply to changes in transition-state solvation. However, we expect the extent of transition-state solvation to be less than that of ground-state solvation; thus, ignoring the transition-state effect will make differences in degree but not in direction.