particularly when applied to bi- and monofunctional inhibitors. If it is assumed that the benzamido group in benzamide can interact with the center ρ_2, it is also necessary to assume that it can interact with the center ρ_1.[4,7] While it is conceivable that benzamide may interact with one center more readily than with the other, both of these intermediate complexes would block combination of the catalytically active site of the enzyme with trifunctional specific substrates such as nicotinyl-L-tryptophanamide and acetyl-L-tyrosinamide. Therefore the experimental value of K_I for benzamide could be a function of two separate dissociation constants, one for each of the above two intermediate complexes. The situation may be even more complicated since the existence of a third complex based upon simultaneous interaction of two molecules of benzamide with the centers ρ_1 and ρ_2 cannot be entirely excluded. Thus the relatively low value of K_I for benzamide may reflect an enhanced probability of combination arising from a multiplicity of modes of combination rather than a high affinity in any particular mode. At present it is not possible to evaluate quantitatively the relative contributions of each of the three types of intermediate complexes that may be formed.

While it may be fortuitous that the K_I value of acetanilide, 10.4×10^{-3} M, is practically identical with that of phenylacetamide, 10.2×10^{-3} M, it does appear that with these two isomeric competitive inhibitors a transposition of the $-CH_2-$ and $-NH-$ groups present in their side chains is without demonstrable effect upon the over-all binding process. This indicates that with these two compounds the principal factor in the combination process is the size and shape of the molecule, and hence a van der Waals interaction.

The data obtained during the course of this investigation have pointed out the existence of two fundamental aspects of certain enzyme-inhibitor and enzyme-substrate interactions involving α-chymotrypsin, *viz.*, (a) the fact that the affinity of the enzyme for certain anionic competitive inhibitors may be profoundly influenced by the nature of the buffer present in the reaction system, and (b) that several different modes of combination may be involved in the interaction of this enzyme with a given specific substrate or competitive inhibitor. Since both of these issues are important for a correct understanding of available kinetic data it is hoped that studies now in progress will expand our knowledge along these lines.

Experimental[21,22]

Competitive Inhibitors.—β-Indoleacetic acid, m.p. 167–168°; β-(β-indole)-propionic acid, m.p. 133–134°; γ-(β-indole)-butyric acid, m.p. 123–124°; benzoic acid, m.p. 122–123°; phenylacetic acid, m.p. 76–77°; β-phenylpropionic acid, m.p. 48–49°; γ-phenylbutyric acid, m.p. 50–51°; acetanilide, m.p. 113–114°; and benzamide, m.p. 129–130°, were Eastman Kodak Co. products which were recrystallized at least twice from appropriate solvents. Phenylacetamide, m.p. 157–158°; β-phenylpropionamide, m.p. 105–106°, and γ-phenylbutyramide, m.p. 84–85°, were prepared from the corresponding acids by ammonolysis of the respective acid chlorides. Ammonolysis of 3 g. of crude methyl β-(β-indole)-propionate, prepared by the reaction of methanolic hydrogen chloride with the corresponding acid, gave 1.5 g. of β-(β-indole)-propionamide, fine stunted needles, m.p. 204–205°.

Anal. Calcd. for $C_{11}H_{12}ON_2$ (188): C, 70.3; H, 6.4; N, 14.9. Found: C, 70.3; H, 6.2; N, 14.9.

Enzyme Experiments.—The methods used were identical with those described previously. The preparation of the principal specific substrate, *i.e.*, nicotinyl-L-tryptophanamide for which $K_S = 2.7 \times 10^{-3}$ M,[10] has also been described.[23] The enzyme preparation was an Armour product, lot no. 90402, and it will be noted that the specific enzyme concentrations E'_S and E'_I for all experiments were such as to satisfy zone A conditions.[10,24,25]

(21) Microanalyses by Dr. A. Elek.

(22) All melting points are corrected.

(23) B. M. Iselin, H. T. Huang, R. V. MacAllister and C. Niemann, THIS JOURNAL, **72**, 1729 (1950).

(24) O. H. Straus and A. Goldstein, *J. Gen. Physiol.*, **26**, 559 (1943).

(25) A. Goldstein, *ibid.*, **27**, 529 (1944).

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF FORDHAM UNIVERSITY]

Effect of Methyl Substitution on the Decarboxylation of Picolinic Acids

BY NELSON H. CANTWELL AND ELLIS V. BROWN

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In an attempt to elucidate the decarboxylation mechanism of picolinic acid, the rates of decarboxylation of picolinic acid itself, four monomethyl derivatives and one dimethyl derivative were studied. The observed reaction rates were found to be first order in all cases. The E and A values of the Arrhenius equation and the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values of the absolute reaction rate theory were then determined. On the basis of the data obtained, mechanisms for the decarboxylation of picolinic acid and the effect of methyl substitution on the reaction are proposed.

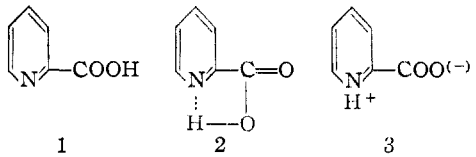
In the process of investigating the mechanism of the Hammick reaction,[1] the necessity of having some knowledge concerning the mechanism of decarboxylation of the acid involved in the reaction became immediately apparent. Picolinic acid, one of the four acids which have been shown to undergo the Hammick coupling upon decarboxylation, was chosen for study. It was thought advisable to determine the rate of the uncatalyzed decarboxylation of picolinic acid and some of its methyl derivatives which might then help to elucidate this process. Uncatalyzed reactions were selected because a report[2] on the rate of breakdown of picolinic acid in various solvents did not agree with the rate as observed during the Hammick reaction.

The question then arose as to what mechanism might be postulated to account for the ease with which picolinic acids and acids similar in structure decarboxylate. One can postulate three reactive

(1) P. Dyson and D. L. Hammick, *J. Chem. Soc.*, 1725 (1937); 809 (1939); 173 (1949); 659 (1949).

(2) H. Schenkel and A. Klein, *Helv. Chim. Acta*, **28**, 1211 (1945).

forms, which could possibly be initial reactants in the process. They are (1) the un-ionized acid, (2) the chelated form and (3) the zwitterion form.



1 2 3

Reactive form (1) can be eliminated as a possibility for it has been fairly well established in the literature[3] on decarboxylation that the initial reactant is an anionic or intramolecular hydrogen bonded structure rather than the free acid except in the case of the dibasic acids oxalic and malonic.[4] Both forms (2) and (3) increase the positive potential of the ring nitrogen and reduce the electron density on the α-carbon which could then exert an attraction on the carbon to carboxy pair of electrons, drawing them toward the ring and favoring release of carbon dioxide.

The second form is suggested in the work of Doering and Pasternak on α-pyridylacetic acids.[5] Similar cyclic intermediates have been proposed by Wiig,[6] Locke[7] and Muus[8] in their work on β-keto acids. The third form is favored by Hammick[9] in his work on quinaldinic, isoquinaldinic and picolinic acids and by Pedersen[10] in his work on β-keto acids.

It can be immediately stated that the cyclic hydrogen bonded structure for the initial reactant seems more probable in the case of β-keto acids than in the case of α-picolinic acids. In the β-keto acids, the cyclic reactant would form a ring of five members excluding hydrogen, whereas, in the case of α-picolinic acid, the ring would be four membered excluding hydrogen. Furthermore, the oxygen of the β-keto acid is much less basic than the nitrogen of the pyridine ring and would have less tendency to form an actual bond with the hydrogen of the carboxy group than would the nitrogen of the pyridine ring.

In order to elucidate the mechanism of decarboxylation of α-picolinic acid, the uncatalyzed reaction rates of picolinic acid and some of its methyl derivatives were determined. If cleavage of carbon dioxide occurred by the heterolysis of the carbon to carboxy bond under the influence of a positive nitrogen, as it would in the case of either of the postulated initial reactants, then methyl groups substituted in various positions on the pyridine should show some effect not only on the rate of decarboxylation but also on the activation energy of decarboxylation.

(3) K. J. Pedersen, *Trans. Faraday Soc.*, **23**, 316 (1927); K. J. Pedersen, *J. Phys. Chem.*, **38**, 559 (1934); E. A. Moelwyn-Hughes, *Proc. Roy. Soc. (London)*, **175A**, 118 (1940); D. Trevich and F. H. Verhoek, THIS JOURNAL, **65**, 1919 (1943); F. H. Verhoek, *ibid.*, **61**, 186 (1939); J. Muus, *J. Phys. Chem.*, **39**, 343 (1935).

(4) A. L. Bernoulli and W. Wege, *Helv. Chim. Acta*, **2**, 511 (1919); R. Fairclough, *J. Chem. Soc.*, 1186 (1938).

(5) V. Z. Pasternak and W. E. Doering, THIS JOURNAL, **72**, 143 (1950).

(6) E. O. Wiig, *J. Phys. Chem.*, **32**, 961 (1928).

(7) A. Locke, THIS JOURNAL, **46**, 1246 (1924).

(8) J. Muus, *J. Phys. Chem.*, **39**, 343 (1935).

(9) D. L. Hammick, *J. Chem. Soc.*, 173 (1949).

(10) K. J. Pedersen, *J. Phys. Chem.*, **38**, 559 (1934); THIS JOURNAL, **60**, 595 (1938).

The decarboxylation rates of picolinic acid and 3-methyl-, 4-methyl-, 5-methyl-, 6-methyl- and 4,6-dimethylpicolinic acids were studied over a temperature range of 20° in hydroquinone dimethyl ether. This solvent was chosen because of its low polar character and high boiling point. The rates were obtained by weighing quantitatively the amount of CO_2 evolved in measured time intervals. All rates were found to be first order. The rate of decarboxylation of picolinic acid was also studied in varied amounts of the solvents used in order to determine whether a change in concentration of solvent would change the rate. The rate remained constant within experimental error as shown in Table I. The E and A values of the Arrhenius equation were then determined and also the $\Delta H^{\pm}$ and $\Delta S^{\pm}$ values from the absolute reaction rate equation

$$k = \kappa \frac{RT}{Nh} e^{\Delta S^{\pm}/R} e^{-\Delta H^{\pm}/RT}$$

The transmission coefficient κ is assumed to be unity.

The rate of decarboxylation of picolinic acid in hydroquinone dimethyl ether was also examined to determine whether heterogeneous catalysis was taking place. Small amounts of glass wool and powdered silica were added but no appreciable change in rate was discovered. Therefore it has been assumed that no heterogeneous catalysis was taking place.

TABLE I

RATES OF DECARBOXYLATION OF PICOLINIC ACID[a] IN CHANGING CONCENTRATIONS OF SOLVENT

Quantity of hydroquinone dimethyl ether, g.	K/sec.$^{-1}$ $\times 10^4$
30	1.99
25	2.01
20	2.11
15	2.15
10	2.11

[a] Five grams of picolinic acid used in each case at a temperature of 171.5°.

Experimental

Preparation of Acids.—Picolinic acid, 6-methyl-, 4-methyl- and 4,6-dimethylpicolinic acids were prepared by method I,[11] in which the substituted 2-picolines were oxidized with $KMnO_4$. 3-Methyl- and 5-methylpicolinic acids were prepared by method II[12] in which the corresponding 2-aminopyridines were diazotized and converted to the bromides and the bromides to the cyanides, which were then hydrolyzed to the acids. The acids were repeatedly recrystallized from anhydrous benzene to constant melting points which agreed with those cited in the literature. Their purity was further verified by neutral equivalents and by collecting the carbon dioxide evolved in a complete decarboxylation. The data are shown in Table II.

The solvent, hydroquinone dimethyl ether, was purified by vacuum distillation in a nitrogen atmosphere. The constant boiling fraction 121° (18 mm.) was used. The ascarite in the absorption tubes was 8–20 mesh and was renewed after each experiment.

Apparatus.—The apparatus consisted of a 125-ml. three-necked flask, equipped with a dry nitrogen inlet tube, a thermometer and reflux condenser. During an experiment the flask was immersed in a constant temperature bath. The reflux condenser was connected to an absorption train, consisting of 2 ascarite absorption tubes, 3 anhydrone dry-

(11) S. Black, E. Depp and B. B. Corson, *J. Org. Chem.*, **14**, 14 (1949).

(12) L. C. Craig, THIS JOURNAL, **56**, 231 (1934).

TABLE II

PHYSICAL CONSTANTS AND DEGREE OF PURITY OF SUBSTITUTED PICOLINIC ACIDS

Acids	M.p. (0°), °C.	Purity by neut.,[a] %	Theor. CO_2 evolved, %
Picolinic	136	99.8	98.2
3-Methylpicolinic	109	99.8	98.8
4-Methylpicolinic	137	99.6	98.1
5-Methylpicolinic	153	99.8	97.5
6-Methylpicolinic	129	99.4	97.9
4,6-Dimethylpicolinic	153	99.5	97.3

[a] One-half gram of the acid in 10 ml. of H_2O was titrated with 0.1 *N* NaOH using phenolphthalein as indicator.

ing tubes and a three-way stopcock, arranged in two sets to alternate. The two absorption (ascarite) and the three drying (anhydrone) tubes were made from glass tubing 100 mm. in length and 15 mm. in diameter, sealed with close fitting one-hole rubber stoppers and fitted with short lengths of glass tubing 5 mm. in diameter. The tubes were connected to one another by short lengths of small bore rubber tubing. The average weight of the ascarite filled absorption tubes at the beginning of each experiment was 30 g. During an experiment they were removed alternately and weighed on an analytical balance to 4 decimal places. Between the three-necked flask and the reflux condenser, a small trap was placed. It served to trap any condensed vapors which rose to the reflux condenser and prevented them from returning to the reaction flask thus affecting its constant temperature.

Procedure.—In each experiment, 0.04 mole of the acid was mixed with 25 g. of the solvent in the reaction flask. The connections were then made and the whole system was swept free of air by a carefully regulated stream of nitrogen. First one absorption tube was exposed and then the other to the nitrogen stream by turning the stopcock in the absorption train. Then the absorption tubes were weighed alternately and this weight recorded as the initial weight. The reaction flask was then lowered into the constant temperature bath and the time necessary to reach the temperature of the bath noted. Carbon dioxide absorbed during this period (usually a very small amount) was weighed and subtracted from that known to have been originally present in the acid. The acid present at zero time, namely the time at which the flask reaches the temperature of the bath, could then be calculated. The absorption tubes were then weighed alternately every 10 minutes. The CO_2 absorbed every 10 minutes was subtracted from that known to have been present in the acid at zero concentration. The logarithm of the decrease of CO_2 in the acid *versus* time was plotted and a straight line was obtained. On the average, ten readings were taken for each experiment and, in all cases, the reaction was allowed to proceed to greater than 85% completion. Reaction rates were taken for each acid over a twenty-degree range at approximately five-degree intervals.

Results

The results presented in Table III were obtained by the use of the first-order equation

$$k = \frac{1}{t} \ln \frac{a}{a - x}$$

for each separate reading of a particular experiment. The k values varied only a few parts in 10^{-7} and the average value was taken as the k representative of that temperature. The a of the equation represents the carbon dioxide initially present in the acid and $a - x$ the amount present at time t. That the rates are of first order is not only shown by an agreement of the k values when calculated for each reading by the equation but also by plotting the logarithm of decrease of CO_2 in the acid *versus* time as shown in Fig. 1.

The heats of activation were calculated by the method of least squares for the five-degree intervals

TABLE III

FIRST-ORDER RATE CONSTANTS FOR THE DECARBOXYLATION OF METHYL SUBSTITUTED PICOLINIC ACIDS

Temp., °C.	k/sec.$^{-1}$ × 10^4	M[a]	Temp., °C.	k/sec.$^{-1}$ × 10^4	M[a]
Picolinic acid			5-Methylpicolinic acid		
171.5	2.155	1.624	175.4	1.510	1.625
179.0	3.942	1.624	181.0	3.625	1.625
184.5	5.866	1.624	185.0	3.675	1.625
190.5	9.176	1.624	189.0	5.232	1.625
3-Methylpicolinic acid			4,6-Dimethylpicolinic acid		
155.5	2.243	1.625	171.0	1.547	1.60
160.0	3.320	1.625	176.5	2.657	1.60
163.0	4.720	1.625	181.0	3.841	1.60
165.5	5.287	1.625	185.8	5.562	1.60
171.2	8.471	1.625	190.5	8.260	1.60
4-Methylpicolinic acid			6-Methylpicolinic acid		
169.6	1.858	1.624	170.0	1.439	1.624
174.5	2.886	1.624	175.1	2.240	1.624
179.5	4.291	1.624	182.1	4.090	1.624
184.8	6.430	1.624	184.0	4.652	1.624
189.5	8.370	1.624	191.0	7.874	1.624

[a] *M*, molality in moles/1000 g. of solvent.

from the lowest temperature for each acid to the highest shown in Table III. The values shown for the heats of activation in Table IV are the average values for 20° range used.

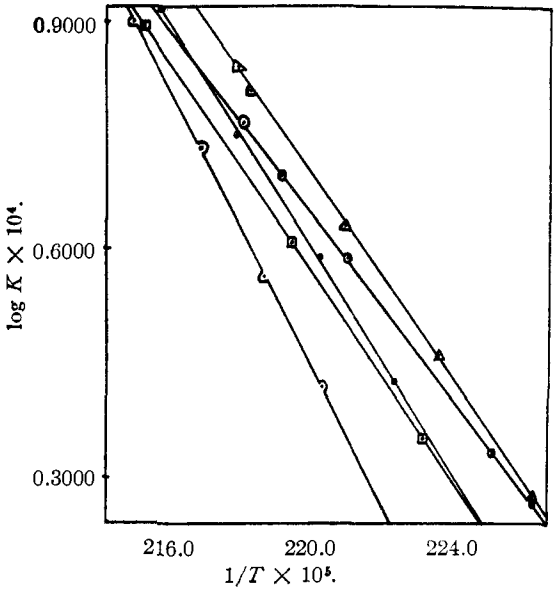


Fig. 1.—⊙, Picolinic acid; ⊡, 6-ME picolinic acid; ◵, 5-ME picolinic acid; ◬, 4-ME picolinic acid; ●, 4,6-DIME picolinic acid.

The $\Delta S^{\ddagger}$ values of activation shown in Table IV were calculated using the mean heat of activation and the corresponding rate constant at approximately 170°, except in the case of 5-methylpicolinic acid where the rate constant at 175° was selected.

In Fig. 2 the logarithms of the reaction rates are plotted *versus* the reciprocals of the absolute temperature for each acid. 3-Methylpicolinic acid does not appear on the graph for its temperature coördinates are off to the right but, if placed on this

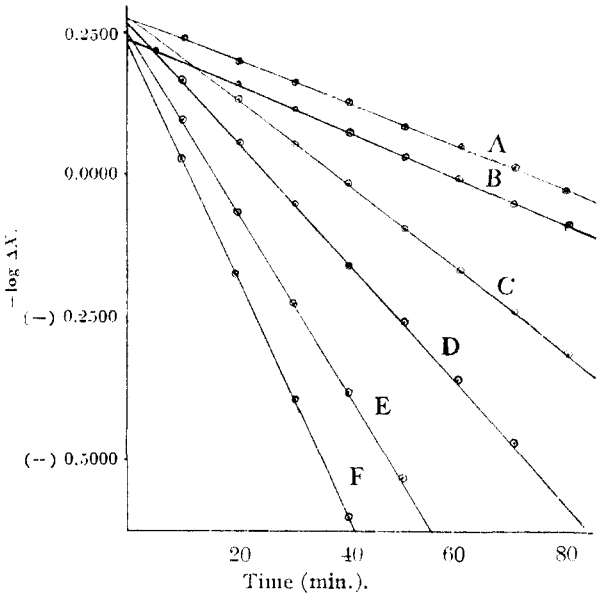


Fig. 2.—A, 5 ME picolinic acid (175.4); B, 4,6 DIME picolinic acid (171); C, 4 ME picolinic acid (174.5); D, 6 ME picolinic acid (182.7); E, picolinic acid (184.5); F, 3 ME picolinic acid (171.2). ΔX = decrease in concn. of acid.

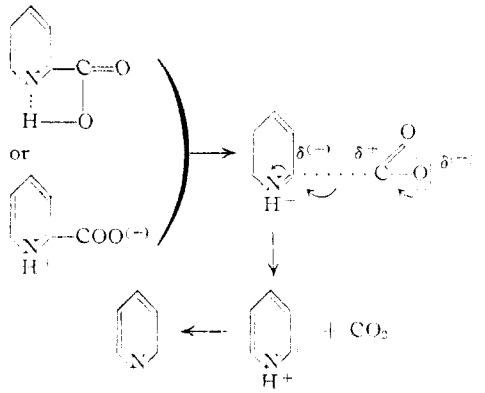
graph, it would appear at the upper right with approximately the same slope as picolinic acid.

TABLE IV

Acid	E, kcal.	$\Delta H^{\pm}$, kcal.	log $10A$	$\Delta S^{\pm}$, cal./deg./mole
Picolinic	31.1	30.2	15.63	− 8.20
3-Methylpicolinic	32.1	31.2	16.66	− 3.09
4-Methylpicolinic	34.6	33.7	17.35	− 0.35
6-Methylpicolinic	35.0	34.1	17.41	− 0.05
4,6-Dimethylpicolinic	38.7	37.8	19.27	+ 8.20
5-Methylpicolinic	40.0	39.1	20.66	+10.30

Discussion

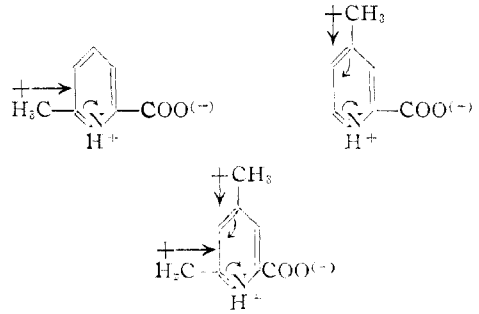
On the basis of the data obtained, it is impossible to decide definitely whether form (2), the cyclic form, or form (3), the zwitterion form, is the predominant initial reactant since both can take part in the mechanism shown.



Both the initial reactants (2) and (3) enhance the positive potential on the ring nitrogen and decrease the electron density on the α-carbon which would facilitate heterolysis of the carbon to carboxy bond.

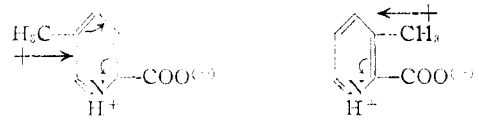
The influence of the methyl groups would be the same on both reactive forms (2) and (3) and can be divided into two separate effects, the first produced by those methyl groups ortho and para to the nitrogen atom and the second produced by those ortho and para to the carboxy group.

In the case of those methyl groups ortho and para to the nitrogen atom, the effect produced as shown, is to induce an electron shift toward the positive nitrogen and thus reduce its pull on the carbon to carboxy electrons.



This would raise the energy necessary for breakdown. The methyl in the 6-position, ortho to the nitrogen, is more effective in this regard than the methyl in the 4-position, as evidenced by the higher heat of activation of the 6-methylpicolinic acid. Moreover, when the two are combined in the 4,6-dimethylpicolinic acid, the heat of activation increases to 37.8 kcal. or about 4 kcal. higher than either one alone, indicating a very decided electron shift toward the nitrogen.

The methyl groups ortho and para to the carboxy group induce an electron shift toward the α-carbon, thus increasing the electron density on it and offsetting the opposite effect produced by the nitrogen atom.



This is particularly effective in the case of the 5-methylpicolinic acid for its activation energy of decarboxylation is 40 kcal. but in the 3-methyl derivative, where we would expect the same effect only greater, the activation energy drops to 32 kcal. This fact shows the peculiarities of the ortho effect.

The increase in log A factor with increased activation energy has been observed by Bernoulli and Wege[13] in their work on substituted malonic acid, by Fairclough[14] in his work on halogen substituted acetic acids and recently by Blomquist and Bernstein[15] in the decomposition of peresters.

NEW YORK 58, N. Y.

(13) A. L. Bernoulli and W. Wege, *Helv. Chim. Acta*, **2**, 511 (1919).
(14) R. A. Fairclough, *J. Chem. Soc.*, 1186 (1938).
(15) A. T. Blomquist and I. A. Bernstein, THIS JOURNAL, **73**, 5546 (1951).