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Aminomalonic Acid. Spontaneous Decarboxylation and Reaction with 5-Deoxypyridoxal*

John W. Thanassi

ABSTRACT: The spontaneous decarboxylation of aminomalonic acid has been studied as a function of pH at constant temperature and ionic strength. It has been found that the neutral species, $^{+}NH_3CH(COOH)COO^{-}$, is about 2.5 times more reactive than the protonated form, $^{+}NH_3CH(COOH)_2$, and approximately 3600 times more reactive than malonic acid monoanion, $CH_2(COOH)COO^{-}$ An anionic mechanism for the spontaneous decarboxylation is suggested. The interaction of aminomalonate with 5-deoxypyridoxal in the pH range 2.9–6.7 at 30° results in the formation of β-(2,5-dimethyl-3-hydroxypyridyl-4-)serine by an aldol-like condensation and decarboxylation. At zero buffer concentration (extrapolated), this appears to be the only reaction. However, in the presence of buffers, there is observed in addition to the condensation reaction, buffer catalysis of the 5-deoxypyridoxal-catalyzed decarboxylation of aminomalonate.

There have been a number of isolated investigations concerned with a possible biochemical role for aminomalonic acid. As early as 1914, the possibility was raised that aminomalonic acid might be an intermediate in protein metabolism, especially in the serine–glycine conversion (Knoop, 1914). Shemin (1946), using a double-labeling technique, ruled out this pathway but the theoretical basis for Shemin's experiments was questioned by Ogston resulting in the well-known "3-point attachment" hypothesis for interaction between enzyme and substrate (Ogston, 1948). More recently, several reports have offered suggestive evidence that aminomalonate may play an as yet unidentified role in biological systems. Thus, an aminomalonic decarboxylase has been found in silkworm glands (Shimura *et al.*, 1956), rat liver (Thanassi and Fruton, 1963), and in several microorganisms (Matthew and Neuberger, 1963a). Ketomalonic acid, the α-keto analog of aminomalonic acid, has been found in several different species (Hammen and Lum, 1962; Heick and Stewart, 1964);

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the origin of this compound is not known. Ketomalonate has been shown in several laboratories to be active as an amino group acceptor in transamination experiments but the product of these reactions was never clearly identified (Cohen, 1939; Braunstein, 1939; Green *et al.*, 1945; Nagayama *et al.*, 1958). Aminomalonate has also been studied as a glycine antagonist and has been found to be a potent inhibitor of δ-aminolevulinate synthetase (Matthew and Neuberger, 1963b).

Chemically, aminomalonic acid is an unusually reactive amino acid which decarboxylates nonenzymatically under rather mild conditions (*vide infra*). Its reactivity is known to be greatly enhanced by the presence of vitamin B_6 compounds such as pyridoxal and pyridoxal 5′-phosphate. For example, a rapid release of CO_2 occurs when aminomalonic acid reacts with pyridoxal phosphate (Thanassi and Fruton, 1963). The interaction of diethylaminomalonate and *N*-methylpyridoxal in ethanol apparently leads to a quinonoid structure (Schirch and Slotter, 1966), proposed to be a key intermediate in certain vitamin B_6 catalyzed reactions (Metzler *et al.*, 1954a). Also, the reaction of aminomalonate with various aldehydes resulting in aldol-like condensations at the α-carbon of aminomalonic acid is promoted by pyridoxal phosphate (Matthew and Neuberger, 1963a,b). Finally, from a theoretical point of view, the arguments of Dunathan (1966) concerning reaction specificity in vitamin B_6 dependent enzymes suggest that the study of chemical systems containing aminomalonate and pyridoxal and its analogs might be of some interest (see Discussion).

As part of a general program concerned with the chemistry and biochemistry of aminomalonates we have investigated the spontaneous decarboxylation of aminomalonic acid and its reaction with 5-deoxypyridoxal, a vitamin B_6 analog.

Materials

Preparation of Aminomalonic Acid, Ammonium Salt. Diethyl formamidomalonate (41 g, 0.2 mole, Aldrich) was dissolved in 500 ml of absolute ethanol containing 34 g of HCl gas and the solution was kept overnight at room temperature. After evaporating from ethanol several times to remove excess HCl, the residual aminomalonic ester hydrochloride was dissolved in 400 ml of 2 N KOH. The solution was heated at 100° for 15 min, chilled in ice, and the pH was adjusted to 6 with 30% acetic acid. Two volumes of 95% ethanol was added, and the precipitate was collected, washed with ethanol, and dried thoroughly *in vacuo*. This material was dissolved in water and applied to a column (3 × 112 cm) of Dowex 1-X8 (50–100 mesh, acetate form). After the column was washed with water, the desired product was displaced with 2 N ammonium acetate, precipitated with ethanol, and recrystallized several times from dilute ammonia–ethanol. The yield was 22.9 g (83% of theory). *Anal.* Calcd for $C_3H_8N_2O_4$ (136.1): C, 26.47; H, 5.92; N, 20.59. Found: C, 26.49; H, 5.72; N, 20.51.

Preparation of 5-Deoxypyridoxal. This compound was synthesized by the procedure of Mühlradt and Snell (1967) except that final purification after silica gel chromatography was effected by sublimation and recrystallization from petroleum ether (bp 30–60°). *Anal.* Calcd for $C_8H_9NO_2$ (151.16): C, 63.56; H, 6.00; N, 9.27. Found: C, 63.35; H, 5.77; N, 9.26.

Preparation of β-(2,5-Dimethyl-3-hydroxypyridyl-4-)serine Hydrochloride (β-5-Deoxypyridoxylserine). 5-Deoxypyridoxal (304 mg, 2 mmoles) and ammonium aminomalonate (2.18 g, 16 mmoles) were dissolved in 200 ml of 0.04 M ammonium acetate buffer (pH 5.43) and kept in the dark under nitrogen for 30 min at room temperature. After acidifying to less than pH 1 with concentrated HCl, the solution was applied to a column (1.5 × 100 cm) of Dowex 50W-X8 (200–400 mesh, hydrogen form). The column was eluted with water (260 ml), 1.2 N HCl (660 ml), 2.5 N HCl (1100 ml), and 3.0 N HCl (1100 ml) at a flow rate of 24 ml/hr. The absorbancy of the effluent was measured at 290 mμ; 80% of the absorbancy applied to the column was recovered in the 400–800-ml fraction of the 3.0 N HCl eluent. This fraction was concentrated to dryness at less than 40° to yield 400 mg (76%) of off-white powder which was recrystallized several times from water–isopropyl alcohol and then dried to constant weight *in vacuo* over P_2O_5 under ether reflux. *Anal.* Calcd for $C_{10}H_{15}ClN_2O_4$ (262.70): C, 45.72; H, 5.75; Cl, 13.50; N, 10.68. Found: C, 45.74; H, 5.95; Cl, 13.20; N, 10.50.

The ultraviolet spectrum of this compound is characteristic of vitamin B_6 derivatives in which the 4 substituent is saturated (Metzler *et al.*, 1954b) having absorption maxima at 290 mμ (0.1 N HCl), 245 and 305 mμ (0.1 N KOH), and 320 mμ (pH 7.0). The proton magnetic resonance spectrum is shown in Figure 5.

All other chemicals utilized were reagent grade obtained from commercial sources. Water employed in these experiments was house-distilled water either redistilled from an all-glass apparatus or passed through a Barnstead mixed-bed demineralizer.

Methods

All absorbancy measurements were made on a Beckman DU monochromator in combination with a Gilford Model 2000 multiple-sample absorbance recorder; the temperature of the cell chamber was maintained at 30° by means of a Haake Model F circulator. Spectra were taken manually with readings every 5 mμ. The disappearance of aldehyde at 380 mμ (or the appearance of product at 320 mμ) was recorded after the addition of 10 μl of 0.1 M 5-deoxypyridoxal in methanol to a temperature-equilibrated 1.0 × 1.0 cm cell containing 1.0 ml of 0.05 M aminomalonate in the desired buffer. The contents were mixed by plunging an 8.0 × 9.5 mm quartz insert up and down several times and readings were begun, usually within 10 sec of the start of the reaction. The insert was left in the cell so that the path length was 0.5 mm.

CO_2 evolution was followed in a Warburg apparatus. For the spontaneous decarboxylation of aminomalonate, the main compartment contained 5.0 ml of the desired buffer and the side arm contained either 10 or 20 μmoles of ammonium aminomalonate in 0.1 ml of H_2O. After the vessels were equilibrated at 45°, the contents were mixed and readings were begun. The rate constants for the spontaneous decarboxylation were calculated using the conventional first-order equation, $kt = 2.3 \log CO_{2\infty}/(CO_{2\infty} - CO_{2t})$. For the determination of CO_2 evolution in the presence of 5-deoxypyridoxal, the main compartment contained 2.5 ml of 0.05 M ammonium aminomalonate in the desired buffer and the side arm contained 0.05 ml of 0.05 M 5-deoxypyridoxal in water. The vessels were equilibrated at 30°, zero-time readings

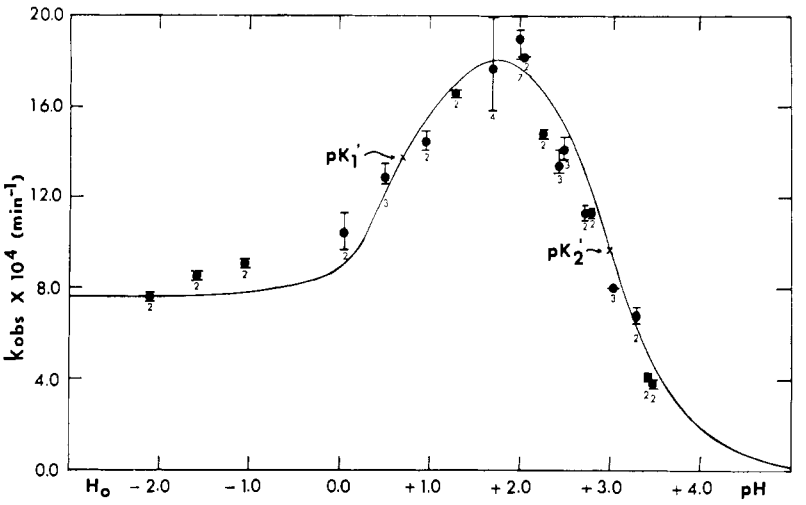


FIGURE 1: The pH dependence of the spontaneous decarboxylation of aminomalonic acid (T = 45°, μ = 0.5 M with KCl except at pH values less than zero). Filled circles are the average of the experimental values with the bars drawn so as to include the entire range of results. Numbers below the bars represent the actual number of determinations. The solid line is calculated from eq 1. Buffer: HCl (−2.1 to +1.0), phosphate (1.6 to 2.8), and formate (2.8 to 3.5).

were taken and then the contents were mixed and the readings were begun. All reactions were run at least in duplicate. The pH of the reaction solutions at the end rarely varied by more than 0.05 pH unit from those at the start. At the lower pH values employed in these experiments, the observed CO_2 evolution was corrected for the spontaneous decarboxylation of aminomalonate by running a reaction solution minus 5-deoxypyridoxal as a control.

pH measurements were made with a Radiometer pHM 26 meter equipped with a GK 2321 C combination electrode thermostatted at 30°. For buffers in the pH region below 1, hydrochloric acid solutions of known H_0 value were used (Leffler and Grunwald, 1963).

Paper chromatography was run ascending on Whatman No. 3MM paper. Solvent systems employed were ethanol–water–diethylamine (70:30:1, v/v) and *t*-butyl alcohol–water–90% formic acid (70:15:15, v/v).

Nuclear magnetic resonance spectra were taken on a Varian A-60 spectrometer. The compounds were lyophilized several times from D_2O in order to remove most of the exchangeable hydrogen atoms.

Results

Figure 1 shows the pH–rate profile for the decarboxylation of aminomalonic acid at 45°. Decarboxylation does not appear to be subject to general catalysis since the rates at two different pH values (2.0 and 3.5) were independent of buffer concentration between 0.1 and 0.5 M, all other conditions being the same. The shape of the curve indicates that the reactive species are the neutral form, AH_2, and the positively charged form, AH_3, the former decarboxylating at a greater rate than the latter. The negatively charged species, AH, is not reactive under the experimental conditions since there was no CO_2 evolution at pH 5.12 (0.5 M acetate buffer). The kinetic expression for the reactions shown in Scheme I is found in

$$v = k_{obsd}(\text{aminomalonic acid})_{total} \qquad (1)$$

where

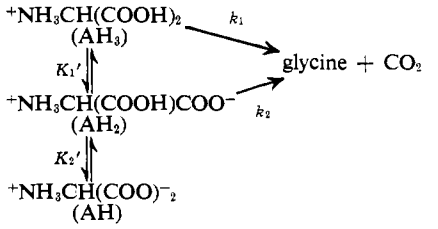
$$k_{obsd} = \frac{k_1(H^+) + k_2K_1'}{(H^+) + K_1'\left(\frac{(H^+) + K_2'}{(H^+)}\right)}$$

eq 1, where k_1 (7.6×10^{-4} min^{-1}) and k_2 (20×10^{-4} min^{-1}) are the respective rate constants for the decarboxylation of AH_3 and AH_2, K_1' (0.2 M) and K_2' (1×10^{-8} M) are the acid dissociation constants, and H^+ is the hydrogen ion activity as determined by the glass electrode at 30°.

K_2' was determined by half-neutralization and is in agreement with the kinetically apparent constant. K_1' is an assigned constant chosen so as to generate the calculated curve shown in Figure 1.

In Figure 2 are shown the experimental data for the rate of CO_2 evolution in acetate buffers at pH 5.12 when 0.05 M aminomalonate is allowed to react with 0.001 M 5-deoxypyridoxal at 30°. Also shown in Figure 2 is a curve (lowest line) at zero buffer concentration which was constructed by extrapolation of the experimental data to zero buffer concentration at selected times and then plotting the extrapolated values *vs.* time. Similar experimental curves were obtained with increasing buffer concentration throughout the entire pH region investigated. It can be seen that in the presence of buffers there is a burst of CO_2 in the initial part of the reaction followed by a leveling off to a slower rate of CO_2 evolution which is constant over the time period studied. Extrapolation of the constant region of the curves to zero time (dashed lines) gives the burst value for a given buffer concentration at a given pH. Extrapolation of the burst values to zero buffer concentration then gives the buffer-independent burst value for that pH, as shown in Figure 3A. Inspection of Figures 2 and 3A indicates that the rate of CO_2 evolution increases with increasing buffer concentration which implies general acid or general base catalysis during the course of the reaction. The constructed curve shown in Figure 2 reveals

SCHEME I



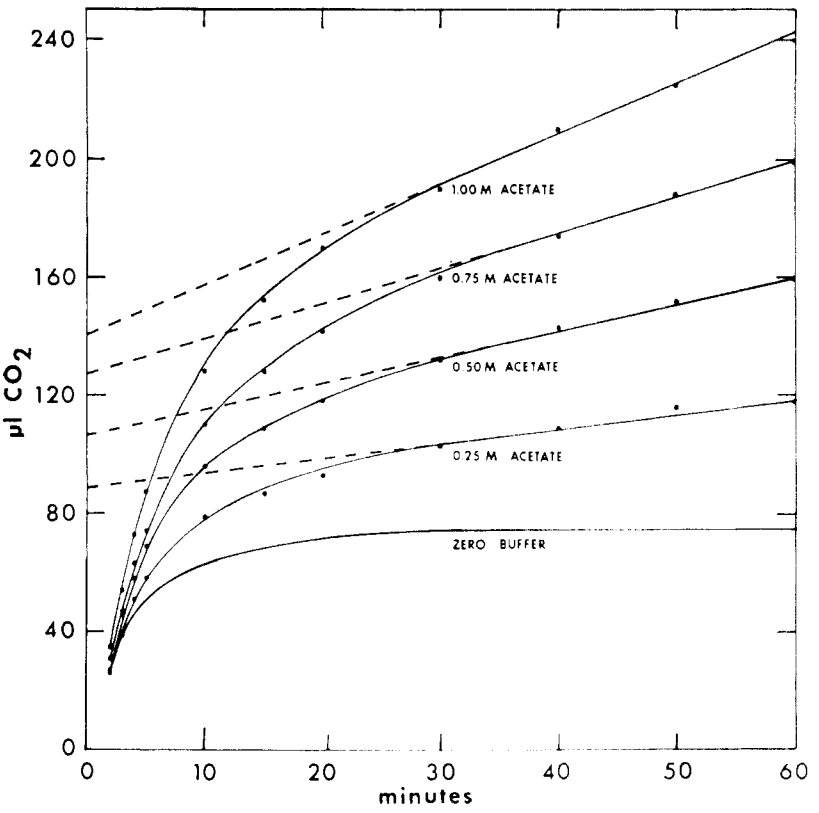


FIGURE 2: Plot of CO_2 released *vs.* time in 2.5-ml reaction solutions containing 1×10^{-3} M 5-deoxypyridoxal and 5×10^{-2} M aminomalonate, pH 5.12, $T = 30°$, $\mu = 1.0$ M (KCl).

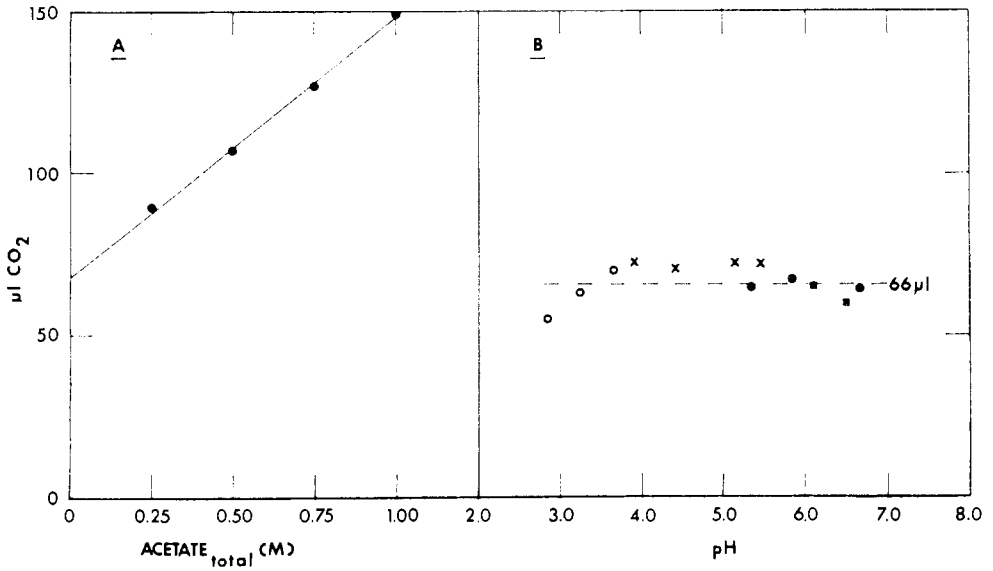


FIGURE 3: CO_2 released. (A) Plot of the amount of CO_2 released in the bursts (ordinate intercepts from Figure 2) *vs.* buffer concentration (conditions as in Figure 2). (B) CO_2 release extrapolated to zero buffer concentration *vs.* pH. Open circles, formate buffers; crosses, acetate buffers; solid circles, phosphate buffers; solid squares, imidazole buffers, $T = 30°$, $\mu = 1.0$ M (KCl).

that, at zero buffer concentration, CO_2 release ceases after the burst and that there is no further slow and constant rate of gas evolution after the initial burst. Therefore, buffer catalysis is *essential* for CO_2 evolution in amounts significantly above that equal to the stoichiometric amount of 5-deoxypyridoxal present in the reaction solution (see below).

Figure 3B is a plot of the buffer-independent burst values between pH 2.85 and 6.70. These burst values, at zero buffer concentration, appear to be fairly constant in this pH range with an average value of 66 μl or 2.9 μmoles of carbon dioxide. It should be noted that the total amount of 5-deoxypyridoxal present in the reaction mixtures was 2.5 μmoles. Therefore,

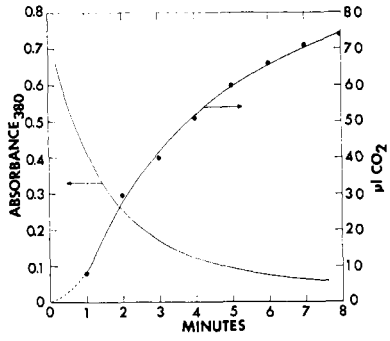


FIGURE 4: Microliters of CO_2 (right ordinate) and absorbancy at 380 mμ (left ordinate) *vs.* time. Conditions as in Figure 2 (0.25 M buffer); path length for absorbancy measurements, 0.5 mm.

on the average, the buffer- and pH-independent values for the amount of CO_2 generated is only about 0.4 μmole above that of the total amount of 5-deoxypyridoxal present in the reaction solution. Reactions were not carried out at pH values higher than 6.70 because the manometric technique used in these experiments becomes limiting owing to CO_2 absorption by alkaline buffers. In the more acid region, the amount of imine formation between pyridoxal derivatives and amino acids becomes extremely small (Metzler, 1957; French *et al.*, 1965).

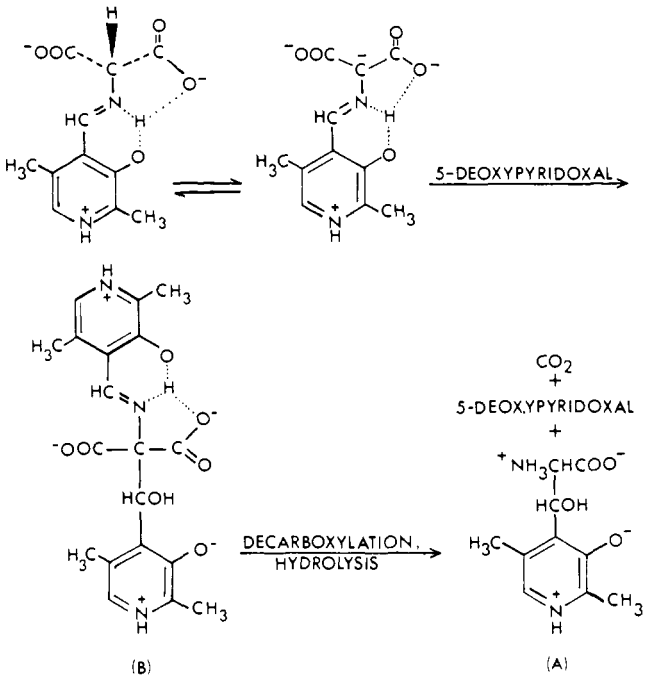
It was observed that the intense yellow color of the reaction solutions bleached during the time of assay and spectrophotometric examination of the reaction solutions confirmed that the 5-deoxypyridoxal was rapidly disappearing. In Figure 4 can be seen plots of the rate of appearance of CO_2 and the rate of disappearance of the aldehyde as measured by the decrease in absorption at 380 mμ under the same conditions. After several hours, the absorbance at 380 mμ had dropped to less than 0.025. Concomitant with the decrease in absorption at 380 mμ is an increase at 320 mμ; these are linearly related to one another. The ultraviolet absorption spectra of the product at several pH values are characteristic of pyridoxal derivatives which have lost the conjugated exocyclic carbonyl group (see Materials).

In order to identify the product of the reaction of aminomalonate and 5-deoxypyridoxal, an experiment on a preparative scale was carried out as described in the Materials section. The ultraviolet and proton magnetic resonance spectra (Figure 5) and the elemental analysis are in agreement with a compound, (A), having the structure shown in Scheme II. This product, β-(2,5-dimethyl-3-hydroxypyridyl-4-)serine, was eluted from a Dowex 50 column in 80% yield in the preparative run.

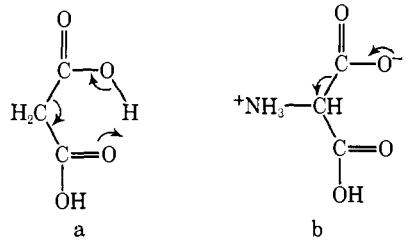
Discussion

Spontaneous Decarboxylation of Aminomalonic Acid. The rate constant at 45° for the decarboxylation of the neutral species, AH_2, of aminomalonic acid is about 2.5 times greater than that for the positively charged species, AH_3. In contrast, the rate of decarboxylation of malonic acid is greater than that of malonic acid monoanion (Fairclough, 1938). Therefore there is a change in the mechanism of decarboxylation between malonate and aminomalonate that can only be attributed to the effects exerted by the positively charged amino group. It is probable that the fully protonated form of malonic acid

SCHEME II



decarboxylates by a concerted cyclic mechanism like that discussed by Westheimer (1959) for the decarboxylation of β-keto acids (mechanism 2a). Removal of a proton from 2a formally introduces a negative charge on either the leaving carboxyl group or the participating carboxyl group. A negative charge on the former no longer allows for a concerted six-membered cyclic mechanism and a negative charge on the latter would exert an inductive effect unfavorable to the electron shifts shown in 2a. Hence one would predict that



(2)

malonic acid would decarboxylate at a greater rate than its monoanion by the Westheimer mechanism. In view of the fact that the neutral species of aminomalonic acid, AH_2, decarboxylates more readily than the positively charged species, AH_3, it appears likely that the favored mechanism for aminomalonic acid decarboxylation is that shown in mechanism 2b. In contrast to the concerted mechanism in 2a, the mechanism of 2b involves the formation of a noncyclic anionic transition state which would be stabilized by the positively charged amino group. This mechanism would explain the fact that the positively charged form of aminomalonic acid, AH_3, is less reactive than the neutral form, AH_2, since protonation of the negatively charged carboxyl group would be expected to inhibit the electron shifts drawn in 2b.

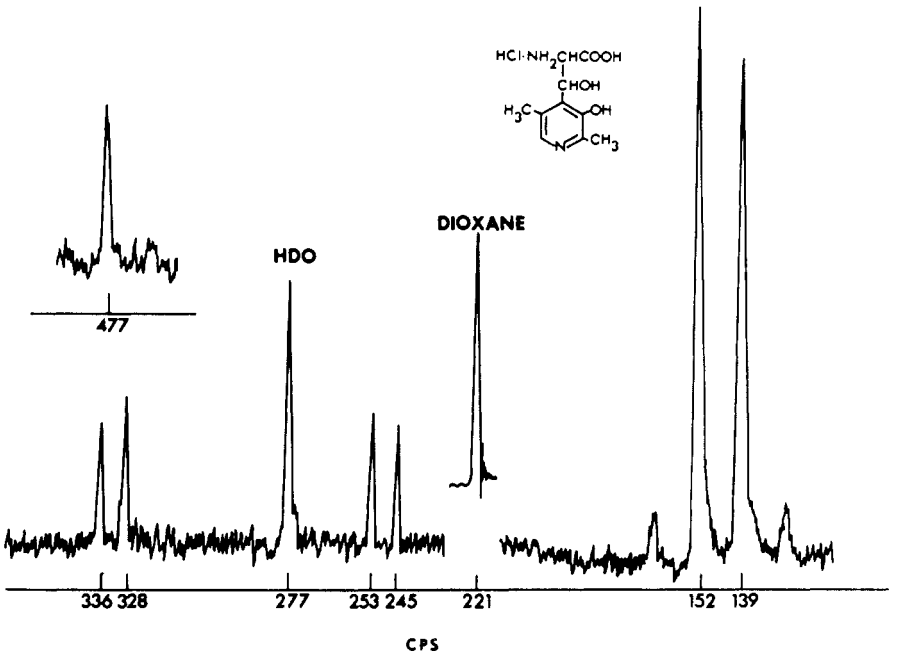
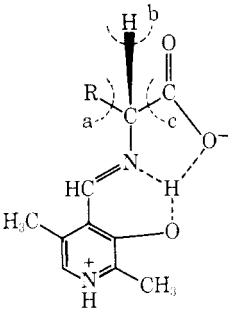


FIGURE 5: Proton magnetic resonance spectrum of β-5-deoxypyridoxylserine in D_2O. Internal standard, dioxane.

The large inductive effect of the positively charged amino group in aminomalonic acid is readily seen on comparing the rate of decarboxylation of the neutral form of aminomalonic acid with the equivalent monoanionic form of malonic acid. The data of Fairclough (1938) for the decarboxylation of sodium hydrogen malonate can be employed to calculate a rate constant of 5.6×10^{-7} min^{-1} at 45°. The corresponding rate constant for decarboxylation of neutral aminomalonate determined in this study is 20×10^{-4} min^{-1}, some 3600-fold greater. The observed rate constants for the decarboxylation of β-keto acids such as oxaloacetic (Kosicki and Kipovac, 1964), acetoacetic (Widmark, 1920), and β,β-dimethyloxaloacetic (Westheimer and Jones, 1941) are of the order of 10^{-3} min^{-1} at temperatures between 25 and 37°. Thus, aminomalonic acid is about as reactive as β-keto acids toward decarboxylation. The inductive effect of the α-amino group is also apparent in the pK_a' values for the carboxyl groups; pK_1', determined kinetically, is approximately 0.7 and pK_2' is 3.0. This makes the first carboxyl group as strong an acid as trichloroacetic and the second carboxyl group about 500 times as acidic as the corresponding carboxyl group in malonic acid which has a pK_a value of 5.7.

Reaction between Aminomalonic Acid and 5-Deoxypyridoxal. Pyridoxal-catalyzed reactions proceed by way of initial Schiff base formation with subsequent labilization of bonds a, b, or c in mechanism 3 leading, among other things, to carbon–carbon bond scission reactions, transamination, and decarboxylation, respectively.[1] Practically all of the enzymatic reactions have been reproduced in relatively simple chemical systems. In general, however, model system studies of pyridoxal catalysis have required elevated temperatures,



(3)

as in the experiments of Snell and his coworkers (Metzler *et al.*, 1954a, and references therein) or fairly long reaction times (Auld and Bruice, 1967, and references therein). In marked contrast, the reactions of aminomalonate with 5-deoxypyridoxal are very rapid and proceed at room temperature. Dunathan (1966) has presented theoretical arguments and there is experimental evidence (Dunathan *et al.*, 1968; Ayling *et al.*, 1968; Bailey *et al.*, 1969) supporting the hypothesis that the bond to be broken in pyridoxal-catalyzed reactions of amino acids must lie in a plane perpendicular to that of the pyridoxal–imine system, *i.e.*, in the position of the hydrogen atom in mechanism 3. Because aminomalonic acid has two equivalent carboxyl groups, this reactive position can be occupied either by a carboxyl group or by a hydrogen atom so that one would expect to observe decarboxylation and/or reactions involving labilization of the α-hydrogen atom (*e.g.*, transamination and/or carbon–carbon condensation reactions). In fact, both decarboxylation and a carbon–carbon condensation reaction occur.

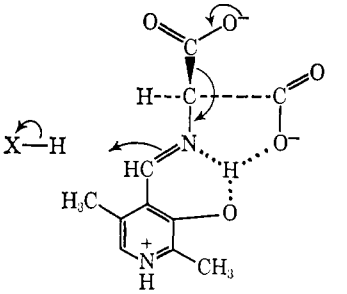
At zero buffer concentration, the reaction between aminomalonic acid and 5-deoxypyridoxal, over the pH range studied, appears to be almost exclusively an aldol-like condensation leading to β-(2,5-dimethyl-3-hydroxypyridyl-

[1] Numerous reviews on the mechanism of vitamin B_6 catalyzed reactions are available. The reader is referred to articles by Snell (1958), Braunstein (1960), and Bruice and Benkovic (1966).

4-)serine (A), according to Scheme II.[2] This conclusion comes from the fact that, at zero buffer concentration, the amount of CO_2 evolved is only slightly more than an amount equal to the stoichiometric amount of 5-deoxypyridoxal present in the reaction solutions and to the fact that β-5-deoxypyridoxylserine was isolated in a preparative run in a yield of 80% (see Experimental Section). A similar preparative experiment using pyridoxal phosphate instead of 5-deoxypyridoxal yielded a nearly quantitative recovery from a Dowex 50 (H^+) column of a compound whose proton magnetic resonance spectrum supported a structure analogous to A in Scheme II. Furthermore, there does not appear to be any appreciable transaminative decarboxylation of the type reported by Kalyankar and Snell (1962) for α-substituted amino acids since examination by paper chromatography of a reaction mixture containing 0.08 M aminomalonate and 0.01 M pyridoxal phosphate in acetate buffer (pH 5.4) showed only very faint traces of an orange ninhydrin-positive spot characteristic of pyridoxamine phosphate; β-5-deoxypyridoxylserine gives a purple color with ninhydrin.

The mechanism suggested for the formation of β-5-deoxypyridoxylserine is presented in Scheme II. The kinetic data presented in Figure 4 support this mechanism. It can be seen that the appearance of CO_2 has a short lag phase and then increases as the aldehyde decreases as measured by the decrease in absorbancy at 380 mμ. This is characteristic of consecutive reactions of the type X → Y → Z (Frost and Pearson, 1961), where X would be the imine plus the imine carbanion formed between 5-deoxypyridoxal and amino malonate, Y would be the adduct B in Scheme II which in this particular case would be a short-lived intermediate and Z would be the product, β-5-deoxypyridoxylserine. Compounds analogous to B in Scheme II have been tentatively identified by paper electrophoresis in reactions involving aminomalonate, pyridoxal phosphate, and various aldehydes (Matthew and Neuberger, 1963a, b). Therefore, in the absence of buffer, the favored reaction in this system is the 5-deoxypyridoxal-catalyzed labilization of the α-hydrogen of aminomalonate followed by an aldol-like condensation on a second molecule of 5-deoxypyridoxal to yield B. This compound would be expected to be very sensitive to decarboxylation owing to the strongly electronegative influence of the pyridoxal moieties in the molecule.

Figure 2 shows that at the highest concentration of acetate buffer, the burst value for CO_2 is 150 μl (6.7 μmoles) and the amount of CO_2 evolved after 60 min is 240 μl (10.7 μmoles). These values are significantly above the 2.5 μmoles that could be formed if the reaction went exclusively by the pathway shown in Scheme II. It is evident, therefore, that the buffer exerts general catalysis in such a fashion that 5-deoxypyridoxal-promoted decarboxylation of aminomalonic acid occurs resulting in the formation of CO_2 and glycine.[3] *A priori*, one would predict buffer catalysis of this reaction to be general acid in nature. The argument in support of this statement is based on results obtained from the study of transamination reactions in model systems which have shown that these reactions can be catalyzed by general base (Thanassi *et al.*, 1965; Auld and Bruice, 1967), general acid (Banks *et al.*, 1961), and concerted general acid–general base catalysis (Bruice and Topping, 1963). These observations are expected since the prototropic shift for the transamination reaction requires pulling a proton in the rate-limiting step from the α-carbon of the amino acid (general base catalysis) and putting a proton on the aldehydic carbon of the aldimine to yield a ketimine (general acid catalysis). If one makes the reasonable assumption that the rate-limiting step in the decarboxylation reaction is cleavage of the carbon–carbon bond (mechanism 4), then general catalysis by acids, XH, is predicted. Unfortunately, no experimental evidence is offered in support of this prediction. The sophisticated kinetic studies of Bruice



(4)

and coworkers (Auld and Bruice, 1967, and references therein) have shown that one needs to know or be able to estimate accurately the rate and equilibrium constants for imine formation from amino acid and aldehyde as well as the acid dissociation constants for the various imine species in order to determine the nature of general buffer catalysis. The very rapid rates of reaction in this study preclude a detailed analysis since imine formation cannot be examined independently of subsequent reactions. Nevertheless, buffer catalysis, whatever its nature, markedly enhances CO_2 release in this system and therefore increases the ratio of decarboxylation to aldol-like condensations, *i.e.*, it imparts specificity to the reaction. This is similar to the observation that general bases specifically catalyze transamination reactions in model systems but not competing side reactions (Thanassi *et al.*, 1965).

The slow, constant rate of CO_2 evolution after the burst and in the presence of buffers may be rationalized in the following ways. The condensation step in Scheme II requires two molecules of aldehyde. This bimolecular reaction would become less likely as the reaction proceeded as a result of a continually decreasing concentration of the reactants. Thus, buffer-promoted 5-deoxypyridoxal-catalyzed decarboxylation of aminomalonate may become relatively more important at very low aldehyde concentrations. Alternatively, β-5-deoxypyridoxylserine may be undergoing some retro reaction to yield glycine and regenerate 5-deoxypyridoxal which would then be available in an equilibrium process for catalysis of decarboxylation of aminomalonic acid. The latter possibility can be eliminated, however, since a manometric assay mixture (2.5 ml) which was 0.05 M in aminomalonate and

[2] Metzler *et al.* (1954b) have isolated a compound in which the 5-methyl group in A of Scheme II is replaced by a hydroxymethyl group, after heating a reaction mixture containing glycine, pyridoxal, and alum. They have given this compound the trivial name β-pyridoxylserine. By analogy, product A in Scheme II can be called β-5-deoxypyridoxylserine.

[3] Glycine was identified by paper chromatography after excess aminomalonate was removed by passing a preparative scale reaction mixture through a column of Dowex 1 (acetate form).

0.001 M in β-5-deoxypyridoxylserine in 1.0 M acetate buffer (pH 5.12, 30°) showed no significant evolution of CO_2.

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