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Vitamin B_6 Catalysis. α-Phenylaminomalonate-Catalyzed Reactions of 5-Deoxypyridoxal†

John W. Thanassi

ABSTRACT: The pH–rate profile for the decarboxylation of α-phenylaminomalonic acid has been determined and describes a bell-shaped curve. The reactive species is the neutral, zwitterionic form, $+NH_3C_6H_5(COOH)COO^-$, which decarboxylates approximately 5×10^6 times more readily than malonic acid monoanion, $CH_2(COOH)COO^-$. The products from the reaction of α-phenylaminomalonate and 5-deoxypyridoxal at pH 5.2 were separated by ion-exchange chromatography and were identified as 5-deoxypyridoxamine, a dimer incorporating one molecule of 5-deoxypyridoxamine and one molecule of 5-deoxypyridoxal (compound J), and a diastereoisomer of compound J (compound K). The reactions of 5-deoxypyridoxal with aminomalonic acid, α-methylaminomalonic acid, and α-phenylaminomalonic acid are compared, and discussed in terms of the mechanism of vitamin B_6 catalysis, with reference to electronic and steric control factors.

Aminomalonic acid, $NH_2CH(COOH)_2$, and its derivatives are interesting compounds on both chemical and biochemical grounds. This amino acid was utilized by Ogsten (1948) to illustrate his theoretical arguments that enzymes can in fact distinguish groups on apparently symmetrical, optically inactive, molecules of the type C (a,b,d,d). Ogsten's publication led to greater insights on the stereochemistry of enzyme-catalyzed reactions and had the important consequence of reinstating citric acid as an intermediate in the tricarboxylic acid cycle of glucose oxidation. A possible biological role for aminomalonic acid as an intermediate in the serine to glycine conversion was proposed about 60 years ago by Knoop (1914) and was later explored by Shemin (1946). More recently, aminomalonic decarboxylase activity from silkworm glands (Shimura *et al.*, 1956) and rat liver (Thanassi and Fruton, 1963) has been reported, leading to further speculation on potential roles for aminomalonic acid in intermediary metabolism (Meister, 1965). However, the possibility of a biological role for aminomalonic acid appears to have been laid to rest in a recent publication by Palekar *et al.* (1973) who demon-

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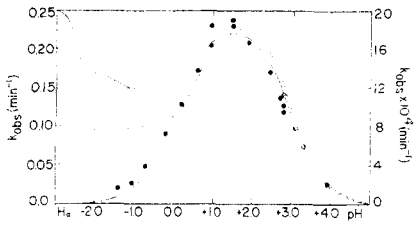


FIGURE 1: pH–rate profiles for the decarboxylation of α-phenylaminomalonate (solid line and left ordinate), aminomalonic acid (dashed line and right ordinate), and malonic acid (insert). Filled circles are the experimental data for α-phenylaminomalonate (T = 30°, μ = 0.50 except at pH values below zero). The solid line is calculated from eq 2. Buffers used are: HCl (+1.4 to −1.0), phosphate (0.5–2.8), formate (2.8–3.4), acetate (3.9–4.5).

strated that the aminomalonic decarboxylase activity found in rat liver is associated with the enzyme serine hydroxymethylase.

Chemically, aminomalonate derivatives are inherently reactive molecules. For example, in marked contrast to malonic acid itself, or other amino acids, decarboxylation of aminomalonates occurs under very mild conditions (Thanassi, 1970, 1971, 1972). The dinitrile of aminomalonic acid, $NH_2CH(CN)_2$, is a particularly interesting compound because of its implication as an intermediate in the chemical evolution of biologically important molecules (Ferris and Orgel, 1965; Sanchez *et al.*, 1967). Indeed, as early as 1914, Johnson and Nicolet (1914) investigated diethylaminomalonate and aminomalononitrile as precursors for purines and pyrimidines.

In chemical systems containing pyridoxal and its analogs, aminomalonates have been shown to be extremely sensitive towards B_6-catalyzed reactions and have provided insight into the mechanisms of pyridoxal-catalyzed reactions (Abbott and Bobrik, 1973; Thanassi and Fruton, 1963; Thanassi, 1970, 1972). Previous communications from this laboratory have dealt with the 5-deoxypyridoxal-catalyzed reactions of aminomalonic acid (Thanassi, 1970) and α-methylaminomalonic acid (Thanassi, 1972). As a systematic and logical extension of these studies, and as an entry into the investigation of steric and electronic effects on pyridoxal catalysis, we have prepared α-phenylaminomalonic acid and have examined its spontaneous decarboxylation and its reactions in the presence of 5-deoxypyridoxal.

Experimental Section

Materials

Preparation of α-Phenylaminomalonic Acid, Ammonium Salt. Diethyl α-phenylaminomalonate was synthesized from chloroamine and diethyl phenylmalonate by the procedure of Horiike *et al.* (1969) and isolated as the hydrochloride (mp 130–133°). *Anal.* Calcd for $C_{13}H_{18}NO_4Cl$ (mol wt 287.7): C, 54.26; Cl, 12.32; H, 6.30; N, 4.87. Found: C, 54.54, Cl, 12.05; H, 6.45; N, 4.73.

The diester was saponified with KOH, and α-phenylaminomalonic acid was isolated in 85% yield as the monoammonium salt employing the ion exchange procedure previously described for the preparation of the ammonium salt of α-methylaminomalonic acid (Thanassi, 1972). The product was recrystallized from water–ethanol. The air-dried product was stored in the freezer. A proton magnetic resonance (pmr) spectrum in D_2O indicated that the compound as isolated contained one ethanol of solvation. Decarboxylation of a sample in acid yielded the calculated amount of CO_2 and the sole product of the reaction was D,L-α-phenylglycine. *Anal.* Calcd for $C_9H_{12}N_2O_4 \cdot C_2H_5OH$ (mol wt 258.3): C, 51.15; H, 7.02; N, 10.84. Found: C, 50.78; H, 6.90; N, 11.19.

Chemicals. 5-Deoxypyridoxal was from previous experiments (Thanassi, 1970). D,L-α-Phenylglycine and diethyl phenylmalonate were obtained from Eastman and Aldrich, respectively. D_2O (99.8%) and AG50W ion exchange resin were purchased from Bio-Rad. All other chemicals were reagent grade, purchased from commercial suppliers. Water employed in these experiments was house distilled water, redistilled in an all-glass apparatus.

Reaction between 5-Deoxypyridoxal and α-Phenylaminomalonic Acid; Isolation of Fractions I–IV. 5-Deoxypyridoxal (78 mg, 0.5 mmol) was dissolved in 50 ml of 0.04 N ammonium acetate buffer at pH 5.2. To this solution was added 377 mg (1.5 mmol) of monoammonium α-phenylaminomalonate. The reaction was allowed to proceed for 3 hr in the dark under nitrogen at room temperature. After acidification with concentrated HCl to a pH below 1, the solution was degassed under a vacuum, and then applied to a 1.5 × 33 cm column of AG50W X8 (200–400 mesh) cation exchange resin in the hydrogen form. The column was eluted under a constant pressure head (Marriott flask) in a stepwise fashion with 2.4 N HCl (45 fractions), 2.8 N HCl (45 fractions), 3.2 N HCl (35 fractions), and 3.6 N HCl (110 fractions); the flow rate was 55–60 ml/hr and fractions were collected every 15 min. The effluent absorbancies at 295 and 255 nm were measured. The tubes containing the individual fractions designated I–IV in Figure 2 were combined, and the separate, pooled tubes were concentrated to dryness on a rotary evaporator at a bath temperature not exceeding 40°. Fractions I, II, and III were identified, by comparison with authentic samples, employing combinations of the following techniques: pmr spectra, elution volume on cation exchange chromatography, thin-layer chromatography (three different solvent systems), mass spectra, and ultraviolet spectra (see Results).

Fraction IV was recrystallized from aqueous acetone and its pmr and chemical ionization mass spectra were obtained. The ratio of the total absorbancies (295 nm) of fractions III–IV (Figure 2) was 1.55. In another experiment, this ratio was 1.38. The total recovery of absorbancy units at 255 and 295 nm applied to the column was greater than 87%, in fractions I–IV.

An experiment identical with the one described above was carried out, substituting α-methylaminomalonate for α-phenylaminomalonate. The products recovered after elution of the AG50W column were identical with those obtained previously (Thanassi, 1972). In addition, a small peak corresponding to fraction IV, above, was eluted with 4.0 N HCl. The total recovery of absorbancy units (295 nm) applied to the column in this experiment was 97%. The ratio of the total absorbancies of the fractions corresponding to III and IV above was 8.0 (III/IV).

Methods

Thin-layer chromatography was performed on Eastman Chromagram sheets containing a fluorescent indicator. The solvent systems employed were: 1-propanol–concentrated NH_4OH–H_2O (6:3:1, v/v), 1-butanol–acetic acid–water (4:1:1, v/v), and 1-propanol–water (7:3, v/v). Authentic samples were run as markers and spots were visualized both by their fluorescence under ultraviolet light and by development with ninhydrin.

Proton magnetic resonance and ultraviolet spectra, CO_2 evolution, and measurements of pH were all carried out as

described previously (Thanassi, 1970, 1972). Elemental analyses were kindly performed by W. C. Alford, and chemical ionization mass spectra were provided by Drs. K. L. Kirk and H. M. Fales.

Results

Spontaneous Decarboxylation of α-Phenylaminomalonic Acid. In Figure 1 are shown pH–rate profiles for the decarboxylation of α-phenylaminomalonic acid, aminomalonic acid, and malonic acid. α-Phenylaminomalonic acid has a bell-shaped profile (solid line) and the reactive species is the species with a net neutral charge, $^+NH_3CC_6H_5(COO^-)(COOH)$ (see Scheme I).

SCHEME I

$^+NH_3CC_6H_5(COOH)_2$ (AH$_3$)

K_1'

$^+NH_3CC_6H_5(COOH)(COO^-)$ (AH$_2$) $\xrightarrow{k_r}$ D,L-α-phenylglycine + CO_2

K_2'

$^+NH_3CC_6H_5(COO^-)_2$ (AH)

K_3'

$NH_2CC_6H_5(COO^-)_2$ (A)

The kinetic solution for the scheme shown in Scheme I leads to eq 1. At the pH values employed in these experiments, $K_3' \ll H^+$ and eq 1 reduces to eq 2, where $k_r = 0.23$

$$v = k_{obsd}[\alpha\text{-phenylaminomalonic acid}]_{total} \quad (1)$$

where

$$k_{obsd} = k_r\left[\left(\frac{H^+ + K_1'}{K_1'}\right) + \frac{K_2'}{H^+}\left(\frac{H^+ + K_3'}{H^+}\right)\right]^{-1}$$

$$k_{obsd} = k_r\left[\frac{K_1'H^+}{H^+(H^+ + K_1') + K_1'K_2'}\right] \quad (2)$$

min^{-1}, $K_1' = 1.0$ M, $K_2' = 1 \times 10^{-3}$ M, and H^+ is the hydrogen ion activity as measured by the glass electrode at 30°. The solid line in Figure 1 is a calculated line constructed from these values and eq 2.

It can be seen that the spontaneous decarboxylation of α-phenylaminomalonic acid has a symmetrical, bell-shaped pH–rate profile in contrast to aminomalonic acid itself which has an unsymmetrical bell-shaped pH–rate profile (dashed line; taken from Thanassi, 1970). It is apparent that α-phenylaminomalonate is very much more sensitive toward decarboxylation than aminomalonate. The rate constant, k_r, is more than two orders of magnitude greater than the rate constant for the decarboxylation of the corresponding neutral species of aminomalonic acid, $^+NH_3CH(COOH)(COO^-)$, without taking into account the difference in reaction temperature (30° for α-phenylaminomalonate, 45° for aminomalonate). As was the case with aminomalonic acid, the decarboxylation of α-phenylaminomalonate does not appear to be subject to general buffer catalysis because the rates of decarboxylation at two different pH values (1.5 and 2.8) were independent of buffer concentration between 0.1 and 0.5 M, all other conditions being identical.

The dimensionless insert in Figure 1 is an assumed pH–rate profile for the decarboxylation of unsubstituted malonic acid; this comes from the experiments of Fairclough (1938) and Bernoulli and Wege (1919) who reported that the fully protonated form of malonic acid is five times more reactive toward decarboxylation than the monoanion of malonic acid.

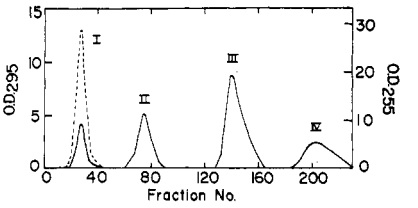
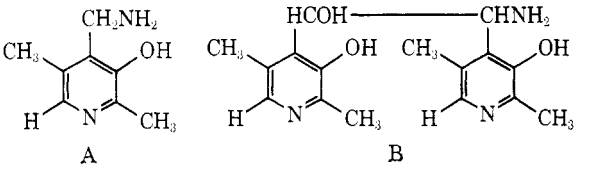


FIGURE 2: Elution pattern of products obtained on AG50W chromatography of a reaction mixture containing α-phenylaminomalonic acid and 5-deoxypyridoxal (pH 5.2). Solid line and left ordinate, absorbancy at 295 nm; dashed line and right ordinate, absorbancy at 255 nm. (The absorbancy at 255 nm of II, III, and IV never exceeded a value of 0.3 absorbancy unit.) Eluent: 2.4 N HCl (fractions 0–45), 2.8 N HCl (fractions 46–90, 3.2 N HCl (fractions 91–125), 3.6 N HCl (fractions 126–235).

In Figure 2 is shown the elution profile obtained upon cation exchange chromatography of a preparative scale reaction mixture containing 5-deoxypyridoxal and α-phenylaminomalonic acid in a molar ratio of 1:3 at pH 5.2 (see Experimental Section). Four fractions were obtained; of the total absorbancy units (295 nm) applied to the column, 87% were recovered in these four fractions.

Fraction I was identified as α-phenylglycine by comparison of its proton magnetic resonance (pmr) spectrum in D_2O with that of an authentic sample, and by the fact that it cochromatographed with authentic α-phenylglycine on thin layer chromatography in three different solvent systems.

Fraction II was identified as 5-deoxypyridoxamine, structure A, by its pmr spectrum in D_2O and by thin layer chromatography in three different solvent systems. A reference sample of 5-deoxypyridoxamine was from previous experiments (Thanassi, 1972).



In a similar fashion, the structure of fraction III was shown by its pmr spectrum and migration on thin layer chromatography to be that of B, a compound previously isolated and identified after the reaction of 5-deoxypyridoxal and α-methylaminomalonic acid, $NH_3C(CH_3)(COOH)_2$, under conditions similar to those used in the present experiments (Thanassi, 1972). The pmr and chemical ionization mass spectra of fraction III are shown in Figures 3a and 4a, respectively.

Fraction IV was the last fraction eluted from the Dowex 50 column. Its pmr and chemical ionization mass spectra are provided in Figures 3b and 4b, respectively. Upon comparison of the pmr spectra of Figures 3a and 3b, it can be seen that the same groups are present in fractions III and IV, *i.e.*, four aromatic methyl groups, two coupled C–H protons, and two aromatic hydrogen atoms. On inspection of structure B, it can be seen that this compound has two adjacent, asymmetric tetrahedral carbon atoms and can exist, therefore, in diastereoisomeric D,L pairs. The extreme similarity of the pmr data and, in particular, the chemical ionization mass spectra of fraction

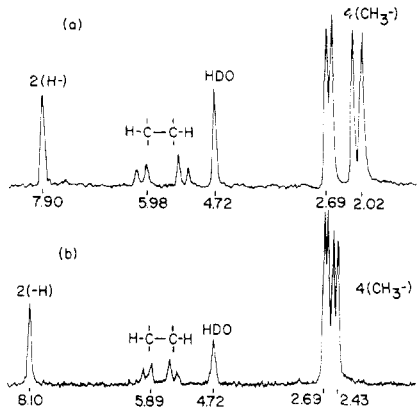


FIGURE 3: Proton magnetic resonance spectra of fraction III (a) and fraction IV (b); spectra were taken in D_2O.

III (Figure 4a) and fraction IV (Figure 4b), confirm that these two compounds are indeed diastereoisomers of each other. The mass spectra of these compounds were obtained consecutively on the same instrument and under the same conditions. They have in common signals at *m/e* values of 286 and 287. Since the molecular weight of B is 303, it is evident that, under the experimental conditions, the quasimolecular ion of mass 304 ($M + 1^+$) is not sufficiently stable to appear in the spectra, and that the signals of 286 and 287 mass units represent the parent ion minus water (mass 18) and ammonia (mass 17), respectively. The common signals in the spectra at *m/e* 151–154 represent the fragments obtained on cleavage of the molecules in half, *i.e.*, between the adjacent, asymmetric carbon atoms. Further loss of ammonia (mass 17) from the species of mass 153 (5-deoxypyridoxamine + 1) leads to the signal of *m/e* 136.

Discussion

Spontaneous Decarboxylation of α-Phenylaminomalonic Acid. α-Phenylaminomalonate is very sensitive toward spontaneous decarboxylation under the conditions of these experiments, the reaction having a half-life of a few minutes at pH values of around 1.5 (Figure 1). Employing the data given by Fairclough (1938) for the decarboxylation of malonic acid

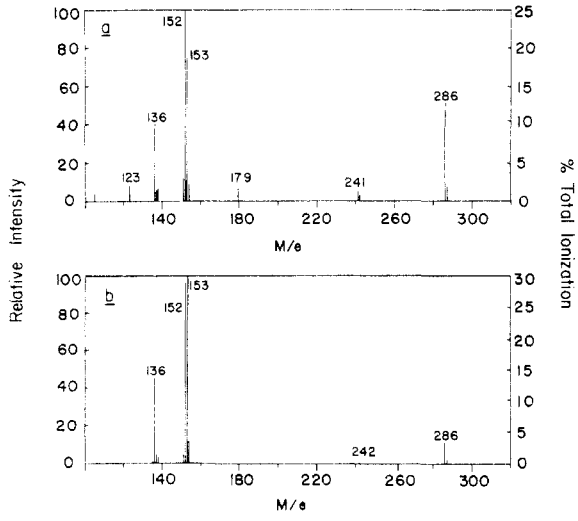
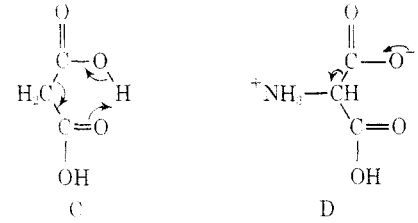


FIGURE 4: Chemical ionization mass spectra of fraction III (a) and fraction IV (b).

monoanion, it can be calculated that at a reaction temperature of 30°, the zwitterionic neutral species of α-phenylaminomalonic acid (AH_2, Scheme I) decarboxylates approximately 5×10^6 times more rapidly than the corresponding monoanionic form of malonic acid, $CH_2(COOH)(COO^-)$. Hence, the α-phenyl and α-amino groups exert very substantial effects on malonic acid decarboxylation.

In addition to this very large rate acceleration, there is a complete change in the mechanism of decarboxylation between malonic acid and α-phenylaminomalonic acid. As shown in Figure 1, the pH–rate profile for the decarboxylation of α-phenylaminomalonate is a symmetrical bell-shaped curve whereas the curve for malonic acid would have two descending sigmoid shaped limbs, proceeding from more acid to less acid solutions. Aminomalonic acid occupies an intermediate position between these two extremes.

These observations lend support to a previous suggestion (Thanassi, 1970) that the more rapid decarboxylation of undissociated malonic acid proceeds by way of a cyclic, concerted mechanism (structure C) of the type originally proposed by Westheimer (1959), whereas the preferential decarboxylation of the zwitterionic, neutral species of aminomalonic acid occurs *via* a noncyclic, carbanionic mechanism (structure D).

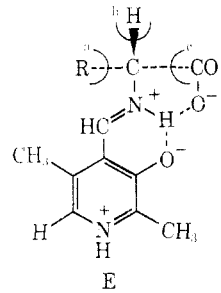


C D

A carbanionic transition state would be significantly stabilized by resonance interaction with the phenyl group in α-phenylaminomalonate and this stabilization would explain both the increased reactivity of α-phenylaminomalonate and the progressive and complete change in mechanism as the α carbon of malonic acid becomes substituted with amino and phenyl groups.

Reaction between α-Phenylaminomalonate and 5-Deoxypyridoxal. Our purpose in studying the 5-deoxypyridoxal-catalyzed reactions of aminomalonates is to examine the influence of substitution on the α carbon of aminomalonic acid on the course of these reactions. This information might provide insight into the mechanisms of catalysis of vitamin B_6 dependent enzymes.

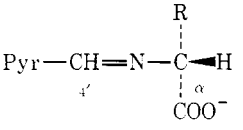
It is clear that there are steric controls operative in vitamin B_6 dependent enzymes. Thus, Dunathan has proposed that the sensitive bond in pyridoxal-catalyzed reactions lies in a plane perpendicular to the extended, conjugated, all-planar pyridoxal–amino acid Schiff base system so as to provide maximal $\sigma-\pi$ overlap during the bond-making and bond-breaking processes; this would be bond b in structure E (Dunathan,



E

1966, 1971). This then provides for reaction specificity; the enzyme evidently is responsible for rotation about the N–C_α bond in the amino acid moiety of the amino acid–pyridoxal Schiff base in order to orient the appropriate bond in the position of bond b, *i.e.*, for transamination reactions (cleavage of C_α–H), bond b would be occupied by hydrogen as shown in structures E, but in decarboxylation reactions (cleavage of C_α–COOH), the carboxyl group would have to be rotated into position b, and so on (Dunathan, 1971; Bailey *et al.*, 1970). (Unlike most amino acids, aminomalonates are extremely sensitive to pyridoxal-catalyzed decarboxylation in chemical systems, and this probably results from the fact that there are two equivalent carboxyl groups so that one can be in the reactive position b at the same time the other is in the stable position c.) These arguments are similar in kind to those advanced by Bruice and Pandit (1960) who showed that the rates of hydrolysis of glutarate and succinate half-esters involving neighboring carboxyl group participation could be greatly enhanced by limiting the number of possible rotamers. In a similar vein, Cohen and coworkers have shown that restriction of rotation of reacting groups (stereopopulation control) can markedly accelerate reactions by orientation effects (Milstein and Cohen, 1969; Borchardt and Cohen, 1972).

In addition to steric control factors involved in the breaking and making of bonds attached to the C_α carbon of the amino acid of the Schiff base, it is clear that there are also steric control factors operative about the carbon atom attached to position 4′ of the hydroxypyridine ring of pyridoxal (the pyridoxal ring in structure F is represented by Pyr). This is apparent



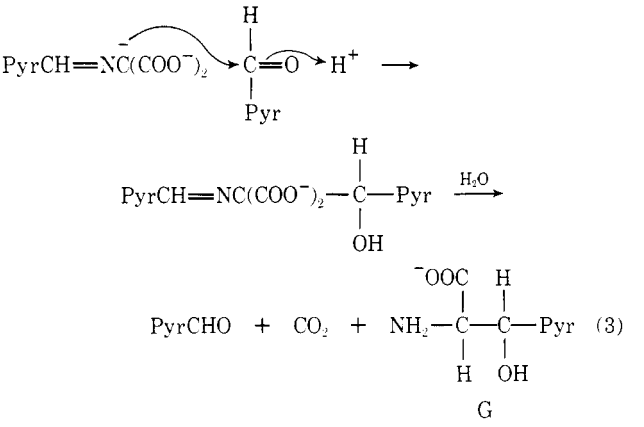
F

from the results of experiments conducted by Dunathan and his collaborators, who found that in transamination reactions there is absolute stereochemical preference in the protonation of the carbon atom designated 4′ in structure F (Dunathan *et al.*, 1968; Ayling *et al.*, 1968; Voet *et al.*, 1973). Evidence was also obtained indicating that an isotopically labeled hydrogen atom removed from C_α of the Schiff base in F can be re-added *via* a stereospecific cis addition to the $C_{4'}$ carbon, generating product pyridoxamine stereospecifically labeled in the 4′-aminomethyl group.

Given the large variety of reactions catalyzed by B_6-dependent enzymes (Snell, 1958; Braunstein, 1960) it is evident that in addition to steric control factors there must be a variety of other devices utilized by enzymes in order to control specificity, stereospecificity, and rates of reactions.

The following discussion deals with the 5-deoxypyridoxal-catalyzed reactions of aminomalonate, α-methylaminomalonate, and α-phenylaminomalonate. In the case of aminomalonic acid, the exclusive product of the reaction with 5-deoxypyridoxal was β-5-deoxypyridoxylserine (G) suggested to arise as shown in eq 3.

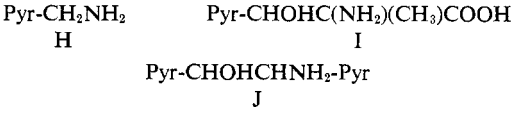
The carbanion in mechanism 3 is probably in steady-state concentration and its existence was inferred because kinetic evidence for the dicarboxylic intermediate in mechanism 3 was obtained (Thanassi, 1970). Therefore, the sole product in the reaction of unsubstituted aminomalonic acid with 5-deoxypyridoxal arises from a condensation reaction that



G

takes place exclusively by a nucleophilic attack of the C_α carbon of the amino acid.

In the case of α-methylaminomalonic acid and 5-deoxypyridoxal, the course of the reaction was very much more complicated and yielded four products, three of which were identified as H, I (probably), and J. Inspection of these products re-

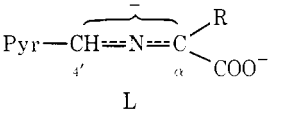


H I J

veals that H and J were both formed by reactions involving, respectively, protonation and condensation at $C_{4'}$ of 5-deoxypyridoxal. Product I, on the other hand, is formed by nucleophilic attack of the C_α of the amino acid. (For the proposed mechanisms for the formation of these products, see Thanassi, 1972.) In addition, the present experiments (see Experimental Section) reveal that a fifth product can be obtained. This compound, K, is formed in small amounts and is a diastereoisomer of J (see below and Results).

In the case of α-phenylaminomalonic acid and 5-deoxypyridoxal, only H, J, and K, the diastereoisomer of J, were formed. All of these products are produced from condensations involving attack of $C_{4'}$ of 5-deoxypyridoxal.

Hence, as the substituent R in structure L changes from



L

COO^- to CH_3 to C_6H_5, the position of condensation changes from 100% C_α, to both C_α and $C_{4'}$, to 100% $C_{4'}$. A variety of factors at the active site of pyridoxal phosphate dependent enzymes must determine the reactive position in the $C_{4'}$–N–C_α system of a vitamin B_6–amino acid Schiff base so as to direct the course of the reaction catalyzed by the enzyme. Among these factors can be assumed to be charge distribution. In the studies reported herein, the substituents can affect the charge distribution in the transition state causing the carbanion to partition toward condensation reactions at C_α, $C_{4'}$, or both. The inductive effect of the methyl group would tend to contribute to condensation reactions at $C_{4'}$ of the 5-deoxypyridoxal component of the Schiff base. In the case of α-phenylaminomalonic acid, condensation at $C_{4'}$ is certainly favored because the phenyl group is directly conjugated to this carbon atom and would substantially contribute to a stabilization of negative charge density about this atom.

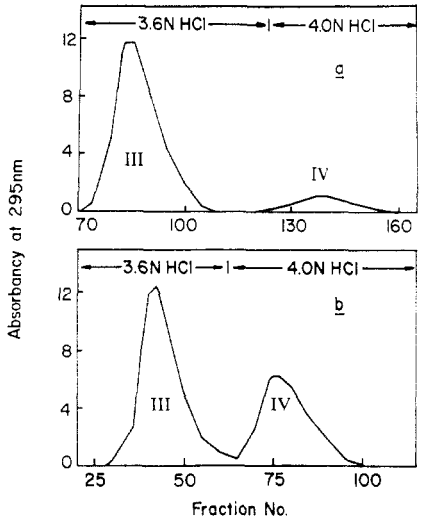


FIGURE 5: Ion-exchange chromatography elution profiles of fractions III (product J) and IV (product K) from reaction mixtures containing 5-deoxypyridoxal and α-methylaminomalonate (a) or α-phenylaminomalonate (b). In a, J/K = 8.0; in b, J/K = 1.5.

In addition to electronic effects, there is an interesting steric control phenomenon occurring in these reactions. This can be seen on comparing the products obtained from the reaction of 5-deoxypyridoxal with α-methyl- and α-phenylaminomalonate under identical conditions (Figure 5). Compound J has two asymmetric carbon atoms and therefore can exist in two diastereoisomeric D,L pairs, J and K. In the reaction of α-methylaminomalonate with 5-deoxypyridoxal the ratio of product J to its diastereoisomeric pair K is 8.0 (Figure 5a). However, in the reaction of α-phenylaminomalonate with 5-deoxypyridoxal, the ratio of J to K is only about 1.5 (Figure 5b). It is evident that the substituents on C_α of the amino acid, which is two atoms removed from the reaction center, exert an influence on the stereochemical course of the reaction, leading to an approximate fivefold difference in the ratio of the formation of the two diastereoisomeric products, J and K, when the substituent is changed from methyl to phenyl.

The result of the replacement of an α-methyl group by a phenyl group, then, is to alter the stereochemical course of the reaction even though neither of these groups appears in the products, K and J.

In summary, both electronic and steric effects are seen in the 5-deoxypyridoxal-catalyzed reactions of aminomalonates. The availability of the α-phenyl derivative of aminomalonic acid opens up a potentially profitable area for systematic investigation. A variety of ring-substituted α-phenylaminomalonates are in preparation in this laboratory and we are investigating these compounds in order to examine how steric and electronic control factors might influence the course of pyridoxal-catalyzed reactions.

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