cis-Diamines as active catalysts for the decarboxylation of oxalacetate[1]

Kenji Ogino,* Hiroki Tamiya, Yoshikazu Kimura, Hideki Azuma and Waichiro Tagaki

Department of Bioapplied Chemistry, Faculty of Engineering, Osaka City University, Sugimoto 3-3-138, Sumiyoshi-ku, Osaka 558, Japan

3-*endo*-Dimethylaminomethyl-1,7,7-trimethylnorbornan-2-*endo*-amine (*cis*-1) and 3-*endo*-dimethylaminomethyl-1,7,7-trimethylnorbornan-2-*exo*-amine (*trans*-1), and the related acyclic 1,3-diamines have been examined for their catalytic activities in the decarboxylation of oxalacetate. The most active catalyst was found to be *cis*-1 involving the Schiff base intermediate. Available evidence indicated that a neighbouring *cis* configuration of two amino groups has dual functions: (1) the activation of the primary amino group by lowering the pK_a for the formation of the Schiff base intermediate at neutral pH, and (2) the stabilization of the Schiff base intermediate by electrostatic and/or hydrogen bonding.

Primary amines catalyse the decarboxylation of β-keto acids through Schiff base intermediates.[2,3] Catalytic activities of simple amines, however, are by no means comparable to those of natural enzymes. In Nature, lysine enzymes[4] such as aldolases[5,6] and acetoacetate decarboxylase[7] catalyse C–H or C–C bond fission by the action of an ε-amino group of lysine at the active site, exhibiting an enormous reactivity. In some of these enzymes, it is known that binding of substrate to one active site almost completely inactivates the other active site, referred to as 'half the site enzymes'.[8] For example, transaldolase is a dimer of two identical subunits each having an active lysine amino acid residue, but active site titration gives only one lysine amino acid residue.[9] Acetoacetate decarboxylase is a dodecamer of twelve identical subunits,[10] but the active site titration gives only six lysine amino acid residues.[11] These examples suggest the possibility that two identical lysine amino acid residues work cooperatively in catalysis, and for a cooperative action two amino groups must be close neighbours.

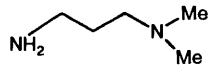
Oxalacetate (OA) is a β-keto acid and its decarboxylation is also catalysed by amines, although the catalysis is carried out by metalloenzymes in Nature, not by a lysine enzyme. In the decarboxylation of OA, it is known that primary amines are the most effective catalysts, secondary amines are much less so, and tertiary amines are ineffective.[2c,2f,4] Catalysis by amines having more than one primary amino group have also been investigated. Ethylenediamine was reported to be a good catalyst for the decarboxylation of OA by Munakata *et al.*[12] Later, a detailed mechanistic study was carried out for the ethylenediamine- and aminoacetonitrile-catalysed decarboxylation of OA by Leussing *et al.*[13] For polyamines, Spetnagel and Klotz reported the catalysis of decarboxylation of OA by modified poly(ethylene imines).[14] More recently, we reported that peraminocyclodextrins in which all C6-OH groups of the parent cyclodextrins had been modified to C6-NH_2 were remarkably active catalysts for the decarboxylation of OA[15] and for aldol condensation.[16] Very recently, Benner *et al.* reported that a rationally designed polypeptide having five lysine residues exhibits the catalytic power of an artificial enzyme for the decarboxylation of OA, and they pointed out the importance of the neighbouring relationship between the amino groups.[17]

The above-mentioned enzyme-catalysed reactions and the polyamine catalysed decarboxylation of OA suggest the importance of neighbouring *cis*-diamine or polyamine structures for the catalysts to be effective in the decarboxylation of β-keto acids. However, for clearer information it is necessary to examine the decarboxylation of OA by using catalysts of more definite structure. In the enolization of aldehydes and ketones, it has been demonstrated by Hine *et al.* that the catalysts having a *cis*-1,3-diamine structure are much more effective than their *trans* counterparts.[18] It was rationalized that a *cis*-1,3-diamine structure is able to form a favourable transition state for enolization that arises from the condensation of the first primary amino group of the catalyst with the carbonyl group of the substrate to form an imine intermediate followed by rate-determining abstraction of α-hydrogen by the second amino group. However, it is important to note that the mechanism of decarboxylation is not the same as that of enolization, since C–C bond fission in the former may not require a base, as does the latter C–H bond fission.

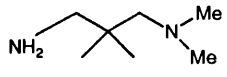
Here we report a study of the decarboxylation of OA catalysed by 3-*endo*-dimethylaminomethyl-1,7,7-trimethylnorbornan-2-*endo*-amine (*cis*-**1**) and 3-*endo*-dimethylaminomethyl-1,7,7-trimethylnorbornan-2-*exo*-amine (*trans*-**2**) and the related acyclic 1,3-diamines (**3** and **4**), in eqn. (1). This investigation

$$^{-}O_2CH_2C(=O)CO_2^{-} + RNH_2 + H^+ \longrightarrow CH_3C(=O)CO_2^{-} + CO_2 + RNH_2 \quad (1)$$

(*cis*)-**1** (*trans*)-**2**



3



4

was initiated to obtain more detailed information for the design of efficient artificial decarboxylases and is a continuation of our previous studies.[1,15,16]

Experimental

Materials

Water for the kinetic studies was purified by deionization followed by double distillation. Buffer reagents for the kinetic experiments were commercially available extra pure materials; buffer (pH), formate (3.2–3.7), acetate (4.0–5.0), MES (5.5–6.6), HEPES (7.0–8.5) and borate (9.0). Organic solvents were purified according to standard methods. Oxalacetic acid (OA), obtained from Sigma, was found to be 98% pure by titration with standard NaOH and used without further purification. 4-Ethyl oxalacetate (4-EtOA) was prepared by saponification of diethyl oxalacetate with KOH according to a literature method:[19] mp 95–98 °C. The two, OA and 4-EtOA, were stored under refrigeration. *N,N*-Dimethylpropane-1,3-diamine (**3**) and *N,N*-dimethyl-2,2-dimethylpropane-1,3-diamine (**4**), obtained from Tokyo Kasei Co., Tokyo, Japan, were purified by distillation. (+)-Camphor was obtained from Wako Pure Chemical Co., Osaka, Japan.

cis-**1** and *trans*-**2** were prepared starting from (+)-camphor according to literature methods.[20–22] The first step, the abstraction of α-hydrogen of (+)-camphor (ref. 20), was modified so as to use lithium isopropylamide. Other procedures were essentially the same as those given in the literature.[23] The crude amines were purified as the benzoates which were then hydrolysed with 6 M HCl to recover the amines as the hydrochlorides: mps were 269–273 °C (lit.,[21] 273–274 °C, decomp.) and 248–253 °C for *cis*-**1**·2HCl and *trans*-**2**·2HCl, respectively. Their structures were fully characterized by their NMR chemical shifts δ_H(JEOL JNM-A400 FT NMR spectrometer; [$^2H_6$]benzene; Me_4Si), *cis*-**1**: 0.77 (s, 3 H), 0.870 (s, 3 H), 0.875 (s, 3 H), 1.06 (br s, 2 H), 1.09–1.17 (m, 1 H), 1.28–1.38 (m, 1 H), 1.43 (m, 1 H), 1.51 (m, 1 H), 1.91 (dd, J = 12.0, J' = 5.8 Hz, 1 H), 2.09 (s, 6 H), 2.21 (m, 1 H), 2.57 (dd, J = 12.0, J' = 9.6 Hz, 1 H), 3.04 (dd, J = 10.8, J' = 1.6 Hz, 1 H); *trans*-**2**: 0.74 (s, 3 H), 0.85 (s, 3 H), 0.98 (s, 3 H), 1.38 (d, J = 5.2 Hz, 1 H), 1.54–1.58 (m, 2 H), 1.71 (d, J = 8.4 Hz, 1 H), 1.87 (dd, J = 12.0, J' = 4.8 Hz, 1 H), 2.10 (s, 6 H), 2.19 (d, J = 11.6 Hz, 1 H), 2.23 (dd, J = 12.0, J' = 8.4 Hz, 1 H), 2.87 (d, J = 11.6 Hz, 1 H), 3.23 (dd, J = 8.4, J' = 4.8 Hz, 1 H).

pK_a Values of the protonated amines were determined at 25 °C by a titration of aqueous solutions of the hydrochlorides with standard NaOH by using an automatic titrator, TitraLab TM11 (Radiometer).

Absorption spectra for both slowly reacting and equilibrium solutions were recorded with a Shimadzu UV-160A spectrophotometer equipped with a controlled temperature cell compartment.

The rates of decarboxylation of OA were followed spectrophotometrically by monitoring the decrease in absorbance of the enol form of OA at 260 nm, according to the method of Spetnagel and Klotz.[14] The sample compartments were thermostatted at 25 °C by means of a circulating water bath. All kinetic data were obtained for fully aqueous solutions. Formate, acetate, Bis-tris, MES and HEPES buffers (0.2 mol dm^{-3}) were used to maintain the desired pH. Each solution contained sufficient KCl to give a final ionic strength of 0.2 mol dm^{-3}. An aliquot from a freshly prepared stock solution of OA was added to the buffered amine solution to initiate the decarboxylation reaction.

Results

pK_a Values of amines

The values determined in this study and those taken from the literature are shown in Table 1. The pK_2 values of dimethylamino groups of 1,3-diamines are not very different from each other, being within the range 9.45–10.13. Similarly, the pK_2 values of 1,2-diamines are also not very different.

On the other hand, a larger difference is seen between the pK_1 values, in both 1,2- and 1,3-diamines. Similar differences are reflected in the ΔpK values, namely, the ΔpK of *cis*-**1** (3.25) is one unit larger than that of *trans*-**2** (2.15), and, similarly, the ΔpK of **4** (3.29) is one unit larger than that of **3** (2.24). A much larger ΔpK (5.7) is seen between the pK_2 values of a bicyclic 1,2-diamine with an unusually low pK_1 (3.0). As discussed later, a lower pK_1 is indicative of a close proximity and a strong inductive effect between the two ammonium groups, as is the case in the bicyclic 1,2-diamine.

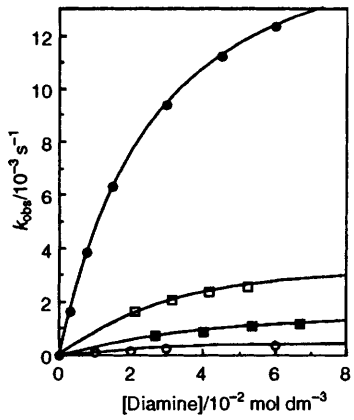


Fig. 1 Plots of pseudo-first-order rate constants (k_{obs}) *vs.* diamine concentrations for the decarboxylation of OA: ●, *cis*-**1**; ○, *trans*-**1**; ■, **3**; □, **4**; [OA] = 3 × 10^{-4} mol dm^{-3}, 25 °C, pH 6.00 (MES, 0.2 mol dm^{-3})

Rates of decarboxylation

According to Spetnagel and Klotz,[14] the absorption maximum at 260 nm is due to the enolic tautomer of OA and this was used as a probe to evaluate the rate of the decarboxylation. The rate of the spontaneous decarboxylation is quite slow (1.1 × 10^{-4} s^{-1} at 37 °C),[24] however, the absorption underwent a first-order decay on addition of amines. The pseudo-first-order rate constants (k_{obs}), thus obtained, were dependent on both amine concentration and pH. In the present study, the kinetic runs were conducted under conditions of an excess of catalyst over the substrate, simply for ease of conducting the experiment,[25] as previously established.[14,15] The decarboxylation occurs completely in the presence of a large excess of the substrate over the amine. As shown in Fig. 1, the rates were dependent on the amine concentrations, giving four curves leading to maximum rate constant values. It is seen in Fig. 1 that the activity is in the order *cis*-**1** > **4** > **3** > *trans*-**2**, and that *cis*-**1** is about five times more active than **4**.

As expected from the fact that amines act as catalysts (C), the saturation curves in Fig. 1 are described by the following equations under conditions of $C_0 \gg S_0$:

$$C + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CS \xrightarrow{k_c} C + P \qquad (2)$$

$$k_{obs} = \frac{k_c C_0}{K_M + C_0}; \; K_M = (k_{-1} + k_c)/k_1 \qquad (3)$$

The Boneni–Hildebrand[6] plots based on eqn. (3) are shown in Fig. 2 from which one obtains k_c, K_M, and k_c/K_M values at pH 6.0; these are listed in Table 2. It can be seen from Table 2 that the k_c values are in the order *cis*-**1** ≫ **4** > **3** > *trans*-**2**, the K_M values are in the order *cis*-**1** < **4** < **3** < *trans*-**2**, and the combined k_c/K_M values are in the order *cis*-**1** ≫ **4** > **3** > *trans*-**2**. In all the parameters, *cis*-**1** is seen to be the most active catalyst of the four amines. As mentioned above, these parameters were all pH-dependent. The pH dependence of k_c, K_M, and k_c/K_M values are shown in Figs. 3, 4 and 5, respectively. It is seen in Fig. 3 that, over the pH range

Table 1 pK_a values of diamines[a]

Diamine	pK_1	pK_2	ΔpK	Ref.
cis-**1**	6.59	9.84	3.25	This work
trans-**2**	7.50	9.45	2.15	This work
3	6.74	10.03	3.29	This work
4	7.67	9.91	2.24	This work
Tetramethyl ethylenediamine	6.50	10.13	3.63	*b*
1,4-Diazabicyclo-[2.2.2]octane	3.0	8.7	5.7	*c*

[a] Determined by titration, 25 °C. [b] *Lange's Handbook of Chemistry*, 11th edn. [c] *Merck Index*, 11th edn.

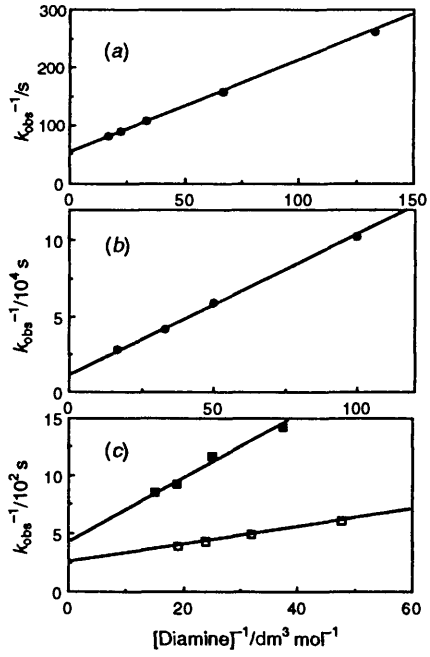


Fig. 2 Boneni–Hildebrand plots of the data in Fig. 1: (*a*), *cis*-**1**; (*b*), *trans*-**2**; (*c*), ■, **3**; □, **4**

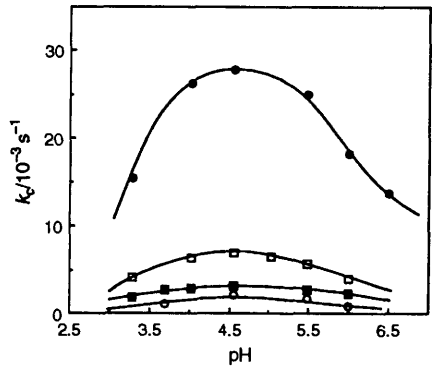


Fig. 3 Plots of pH dependence of K_c values: ●, *cis*-**1**; ○, *trans*-**2**; ■, **3**; □, **4**; 25 °C

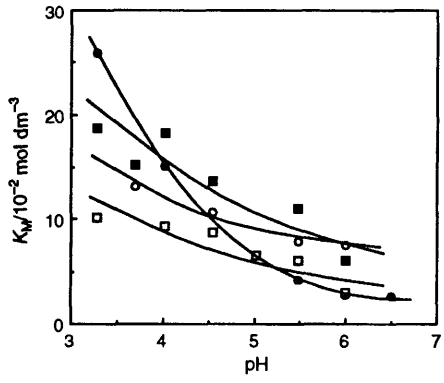


Fig. 4 Plots of pH dependence of K_M values: ●, *cis*-**1**; ○, *trans*-**2**; ■, **3**; □, **4**; 25 °C

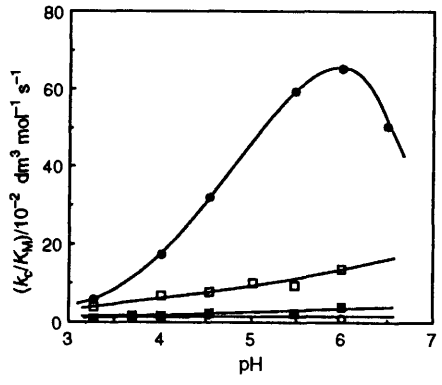


Fig. 5 Plots of pH dependence of k_c/K_M values: ●, *cis*-**1**; ○, *trans*-**2**; ■, **3**; □, **4**; 25 °C

examined, *cis*-**1** shows by far the largest k_c values compared with the other three amines. It is also shown that there is a k_c maximum near pH 4.5, common to all four amines. In Fig. 4 it is shown that the K_M values increase with decreasing pH, indicating that the protonation of amine upon lowering of the pH disfavours the formation of the Michaelis complex. It can also be seen that the K_M values of *cis*-**1** are much smaller than those of *trans*-**2** at high pH (above pH 4.5). Fig. 5 shows that, as expected, the pH effects on k_c/K_M are very different from those shown for k_c in Fig. 3. For *cis*-**1**, the pH maximum is shifted from pH 4.5 for k_c to pH 6 for k_c/K_M. A similar shift is also observed for the other amines. It is important to notice that the activity of *cis*-**1** shown by k_c/K_M becomes even greater than that shown by k_c, compared with those of the other amines near the neutral pH region (~ pH 6).

Enamine formation with 4-ethyl oxalacetate (4-EtOA)

Although difficult in the case of OA because of the decarboxylation, determination of the equilibrium constants for the formation of enamine complexes with amines without decarboxylation is easy for 4-EtOA, and their constants may be compared with the above K_M values. Such a comparison has already been made with poly(ethyleneimine) by Spetnagel and Klotz,[14] and with ethylenediamine by Leussing *et al.*[13] According to their report, a rapid equilibrium was set up accompanied by a strong absorption maximum near 280 nm, which was observed on mixing of an amine with 4-EtOA. This absorption was ascribed to enamine complex.[13] However, the maxima and their intensities appeared to be variable, depending on the nature of amines and the pHs, since the solution is in principle a mixture of imine, enamine, carbinolamine, and their protonated species. Therefore, in this study only overall equilibrium constants K_{EN} were determined for the comparison with the K_M values.

As shown in Fig. 6(*a*), complete complexation was observed by using *ca.* 10 and 50 molar excesses of amine over the substrate with *cis*-**1** and *trans*-**2**, respectively. The absorption

Table 2 Kinetic parameters of diamines for the decarboxylation of oxalacetate[a]

Diamine	$k_c/10^3$ s^{-1}	$K_M/10^2$ mol dm^{-3}	$(k_c/K_M)/10^2$ mol dm^{-3} s^{-1}
cis-**1**	18.2	2.80	65.0
trans-**2**	0.826	7.56	1.09
3	2.28	6.10	3.74
4	3.98	2.98	13.4

[a] [OA] = 3.0 × 10^{-4} mol dm^{-3}; 25 °C, pH 6.00 (MES buffer, 0.2 mol dm^{-3}).

Table 3 Dissociation constants (K_{EN}) of the complexes of 4-EtOA with diamines; 25 °C, pH 7.50 (HEPES, 0.2 mol dm^{-3})

Diamine	*cis*-**1**	*trans*-**2**	**3**	**4**
K_{EN}/mol dm^{-3}	1.01 × 10^{-4}	4.78 × 10^{-4}	7.38 × 10^{-3}	3.86 × 10^{-3}

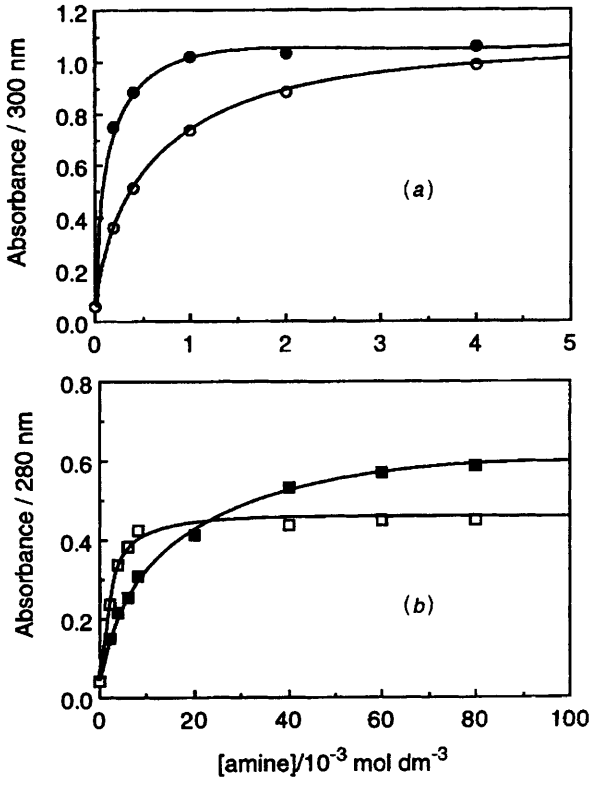


Fig. 6 Absorbance change of mixed solutions of 4-EtOA and amine: [4-EtOA] = 7.5 × 10^{-5} mol dm^{-3}; 25 °C, pH 7.5 (HEPES, 0.2 mol dm^{-3}); (*a*) ●, *cis*-**1**; ○, *trans*-**2**; (*b*) ■, **3**; □, **4**

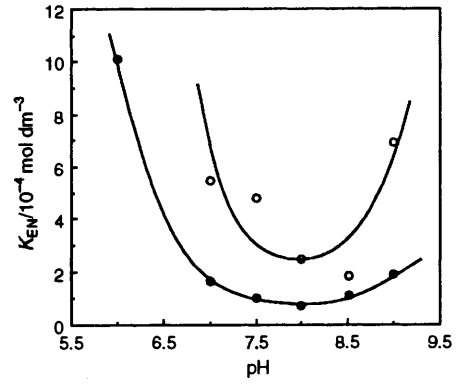


Fig. 7 Plots of pH dependence of K_{EN} values (25 °C): ●, *cis*-**1**; ○, *trans*-**2**

maximum and the extinction coefficient of enamine complex of *cis*-**1** thus observed were 300 nm and 1.34 × 10^4 dm^3 mol^{-1} cm^{-1}, respectively. Fig. 6(*b*) shows that a much higher concentration of the amine was required to attain saturation in the cases of **3** and **4**. Here, the absorption maximum at 280 nm and the intensities at saturation were also different from those in Fig. 6(*a*). At any rate, the Boneni–Hildebrand plots of these saturation curves allow one to calculate the overall equilibrium constants (K_{EN}) at pH 7.50 as summarized in Table 3. The K_{EN} values obtained show that the enamine complexation is more greatly favoured with *cis*-**1** than *trans*-**2**, and with **4** than **3**. The fact that *cis*-**1** has smaller K_{EN} values than *trans*-**2** was also observed for other pH values as shown in Fig. 7, and this trend is qualitatively similar to that observed for the K_M values in Fig. 4.

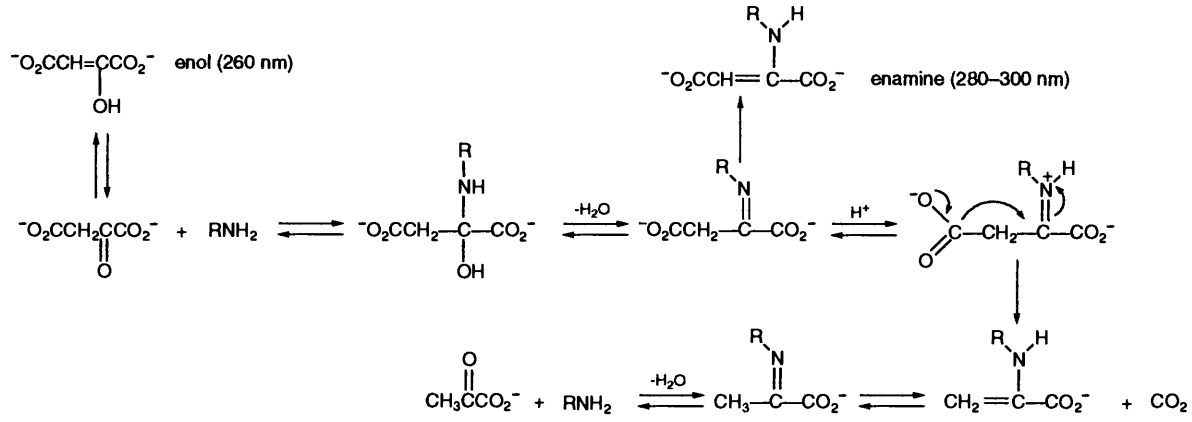
Discussion

As described above, *cis*-**1** isomer is a much more active catalyst than *trans*-**2** for the decarboxylation of OA, as manifested by all parameters k_c, K_M, k_c/K_M and K_{EN}. Similarly **4** is more active than **3**. The present amine catalysis proceeds through the formation of a Schiff base (imine) as shown in Scheme 1,[2,13,14] where the primary amino group is directly involved in the Schiff base formation. The question is then directed to the function(s) of the tertiary amino group in the catalysis.

The first stage is possibly the lowering of the pK_a of the catalytic primary amino group by a charge–charge repulsion between the positively charged ammonium groups, as shown in Fig. 8(*a*). For this function to work effectively, the two amino groups must be close to each other. As expected, pK_1 of *cis*-**1** is about one unit lower than that of *trans*-**2**, and ΔpK is one unit larger in *cis*-**1** than in *trans*-**2**. It is likely that the 2,2-dimethyl groups of amine **4** restricts the free rotation of the C–C bond so as to favour a *cis* conformation of the two amino groups; **3** is more flexible. For acetoacetate decarboxylase, the pK_a of the ε-amino group of the catalytic lysine residue was found to be 6.5 which is four units lower than that of free lysine.[26] In a designed peptide containing five lysine residues, the lowest pK_a of the ε-amino group of lysine was found to be 7.2.[17] We found that peraminocyclodextrins have amino groups of low pK_a.[15] All these facts suggest that the pK_a of one amino group is substantially lowered by the neighbouring amino group(s). The observation that ΔpK_a is one unit larger for *cis*-**1** and **4** compared with *trans*-**2** and **3** is suggestive of intramolecular hydrogen bonding between an ammonium group and an unprotonated amino group in the monoprotonated diamines *cis*-**1** and **4**. An amine having a lower pK_a is thus more active because of this type of interaction at lower pH than one having a higher pK_a in forming an enamine.[4,13]

The second stage is probably acid–base catalysed imine formation, as shown in Fig. 8(*b*). This step itself may not be so important compared with the decarboxylation step in relation to the diamine structure, since previous data have indicated that the former step is much faster than the latter.[13,27] However, it is conceivable that a diamine structure which favours imine formation may also favour decarboxylation as discussed below.

The third factor to be considered is the functions of a protonated tertiary amino group. It may be useful to compare



Scheme 1 Mechanism of amine-catalysed decarboxylation of oxalacetate

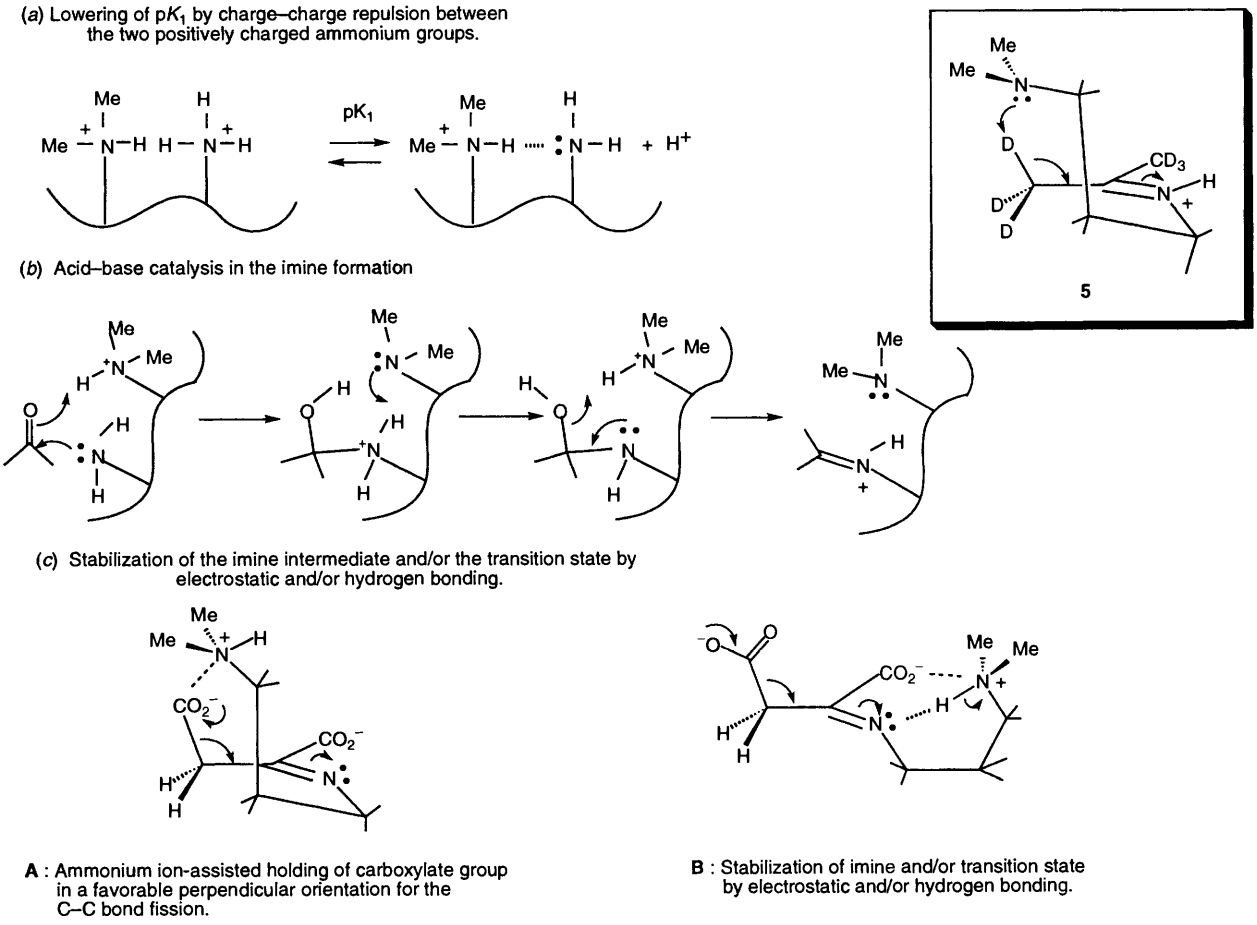


Fig. 8 Possible functions of neighbouring amino groups in the decarboxylation of oxalacetate

the enolization of aldehydes and ketones with the decarboxylation of OA. Hine *et al.* demonstrated that *cis*-**1** is the most effective catalyst among several 1,3-diamines for the dedeuteriation of $(CH_3)_2CDCHO$ or $(CD_3)_2CO$,[23] and this was rationalized by the suggestion that the eclipsed 1,3-diamine structure of *cis*-**1** is the optimum structure for forming an eight-membered transition state, as shown in structure **5**, in which the tertiary amino group can adopt a position for abstraction of the α-deuteron as a general base, thus allowing effective orbital overlap.[18] However, such general-base catalysis is unlikely in the decarboxylation of OA, since its rate-limiting step is C–C bond fission, and this step does not seem to require base-catalysis. This is because the carboxy group to be eliminated is already present as an anion in the pH region in question. Rather, it is important to note that the tertiary amino group exists as an ammonium group over the whole pH region examined. This ammonium group may stabilize the Schiff base intermediate by an electrostatic interaction with either of the two carboxylate anions, as shown in Fig. 8(*c*) (**A** or **B**). In **A**, the ammonium group interacts with the carboxylate group to be eliminated, holding it perpendicular to the plane of C=N double bond, allowing the effective orbital overlap necessary for the decarboxylation. However, such an interaction would, in principle, inhibit C–C bond fission. In **B**, the ammonium group interacts with both the non-departing carboxylate group by an electrostatic interaction and with the nitrogen of the C=N double bond by hydrogen bonding, and these interactions occur in the same plane allowing the C–C bond being cleaved to take up an orientation perpendicular to the plane of the C=N double bond. Similar dual interactions may also be possible for the carboxylate group being cleaved, but they must sacrifice the perpendicular orientation of the C–C bond as in **A**. It is important to note that in **A**, protonation of the nitrogen of the C=N bond is necessary before C–C bond fission, but that it may

occur concurrently in **B** through hydrogen bonding. It might be argued that the very low activity of *trans*-**2** is due to the *exo*-conformation of the 2-amino group, and not simply due to its *trans* structure. This may be an important point for future study, but it should also be noted that *trans*-**2** gave a moderate K_M value and forms a strong enamine complex with a low K_{EN} value.

The K_M and K_{EN} values indicate that their comparison is not straightforward. Since the K_{EN} values can be obtained directly by spectroscopic means, they may reflect the effects of the diamine structure more directly than the K_M values, which are indirect kinetic values. As discussed above, there are reasons for considering the interactions between the carboxylate groups and the tertiary amino group. Therefore, it is no wonder that the effects of the diamine structure on the K_M and K_{EN} values are different because the former are obtained for OA having two carboxylate groups, while the latter are obtained for 4-EtOA having only one carboxylate group.

References

1 Preliminary results have appeared, W. Tagaki, Y. Ohara, Y. Kimura and K. Ogino, *Indian J. Chem.*, 1993, **32B**, 61; W. Tagaki, H. Tamiya, Y. Kimura and K. Ogino, *2nd ISBOC*, 1993, Fukuoka, Japan, Abstracts, p. 192.

2 (*a*) K. J. Pedersen, *J. Am. Chem. Soc.*, 1929, **51**, 2098; (*b*) K. J. Pedersen, *J. Phys. Chem.*, 1932, **38**, 559; (*c*) K. J. Pedersen, *J. Am. Chem. Soc.*, 1938, **60**, 595; (*d*) S. Kaneko, *J. Biochem. (Tokyo)*, 1938, **28**, 1; (*e*) F. H. Westheimer and W. A. Jones, *J. Am. Chem. Soc.*, 1941, **63**, 3238; (*f*) K. J. Pedersen, *Acta Chem. Scand.*, 1954, **8**, 710; (*g*) R. W. Hay, *Aust. J. Chem.*, 1965, **18**, 337; (*h*) J. P. Guthrie, *J. Am. Chem. Soc.*, 1972, **94**, 7024; (*i*) J. P. Guthrie and F. Jordan, *J. Am. Chem. Soc.*, 1972, **94**, 9136; (*j*) K. Taguchi and F. H. Westheimer, *J. Am. Chem. Soc.*, 1973, **95**, 7413.

3 W. P. Jencks, *Catalysis in Chemistry and Enzymology*, McGraw–Hill, 1969; E. E. Snell and S. J. di Mari, *The Enzymes*, 1976, vol. 2, p. 335; A. Fersht, *Enzyme Structure and Mechanism*, 2nd edn., Freeman, 1985.

4 (*a*) D. J. Hupe, in *The Chemistry of Enzyme Action*, ed. M. I. Page, ch. 8, p. 271, Elsevier, 1984; (*b*) D. J. Hupe, in *Enzyme Mechanism*, eds. M. I. Page and A. Williams, RSC, Cambridge, 1987, ch. 17, p. 317.

5 B. L. Horecker, O. Tsolas and C. Y. Lai, *The Enzymes*, 1972, vol. 7, p. 213.

6 O. Tsolas and B. L. Horecker, *The Enzymes*, 1972, vol. 7, p. 259.

7 I. Fridovich, *The Enzymes*, 1972, vol. 6, p. 255.

8 E. Grazi, G. Balboni, K. Brand and O. Tsola, *Arch. Biochem. Biophys.*, 1977, **179**, 131.

9 E. Grazi, P. T. Rowley, T. Cheng, O. Tchola and B. L. Horecker, *Biochem. Biophys. Res. Commun.*, 1962, **9**, 38.

10 W. Tagaki and F. H. Westheimer, *Biochemistry*, 1968, **7**, 895.

11 (*a*) W. Tagaki, J. P. Guthrie and F. H. Westheimer, *Biochemistry*, 1968, **7**, 905; (*b*) M. H. O'Leary and F. H. Westheimer, *Biochemistry*, 1968, **7**, 913.

12 M. Munakata, M. Matsui, M. Tabushi and T. Shigematsu, *Bull. Chem. Soc. Jpn.*, 1970, **43**, 114.

13 D. L. Leussing and N. V. Raghavan, *J. Am. Chem. Soc.*, 1980, **102**, 5635.

14 W. J. Spetnagel and I. M. Klotz, *J. Am. Chem. Soc.*, 1976, **98**, 8199.

15 W. Tagaki, K. Yano, K. Yamanaka, H. Yamamoto and T. Miyasaka, *Tetrahedron Lett.*, 1990, **31**, 3897.

16 W. Tagaki and H. Yamamoto, *Tetrahedron Lett.*, 1991, **32**, 1207.

17 K. Johnsson, R. K. Allemann, H. Widmer and S. A. Benner, *Nature (London)*, 1993, **365**, 530.

18 (*a*) J. Hine and W.-S. Li, *J. Am. Chem. Soc.*, 1976, **98**, 3287; (*b*) J. Hine, *Acc. Chem. Res.*, 1978, **11**, 1.

19 G. Sieber, *Justus Liebigs Ann. Chem.*, 1960, **631**, 180.

20 *Org. React.*, 1954, **8**, 120.

21 Z. Minardi, P. Shenone and P. Farmco, *Ed. Sci.*, 1970, **25**, 519.

22 E. Gelles and R. W. Hay, *J. Chem. Soc.*, 1958, 3673.

23 (*a*) G. Hine and P. K. J. Mookerjee, *J. Org. Chem.*, 1976, **40**, 292; (*b*) J. Hine, *J. Am. Chem. Soc.*, 1980, **102**, 4403.

24 K. J. Pedersen, *Acta Chem. Scand.*, 1952, **6**, 285.

25 The absorption intensity of OA at 260 nm is too large to record at high OA concentration with 1 cm UV cell.

26 P. A. Frey, F. C. Kokesh and F. H. Westheimer, *J. Am. Chem. Soc.*, 1971, **93**, 7266.

27 Our preliminary results based on stopped-flow kinetics indicate that the rate of enamine formation from peramino-β-cyclodextrin and OA is at least ten times faster than that of decarboxylation. K. Yano, Masters Thesis, Osaka City Univ., 1985.

Paper 5/04721H
Received 18*th July* 1995
Accepted 24*th November* 1995