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α-CYANOAMINO ACIDS AND RELATED NITRILES AS INHIBITORS OF GLUTAMATE DECARBOXYLASE

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SUMMARY

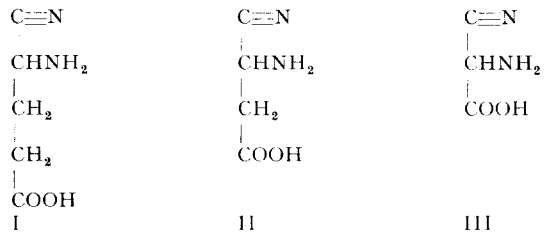
L-γ-Cyano-γ-aminobutyric acid, L-β-cyano-β-alanine, and α-cyanoglycine, new synthetic amino acids with the unique α-cyanoamino acid structure, have been found to be strongly inhibitory to *Escherichia coli* glutamate decarboxylase (L-glutamate 1-carboxy-lyase, EC 4.1.1.15). Eleven related nitriles of simpler structure also are active, which suggests organic nitriles as a new class of enzyme inhibitors. γ-Cyano-γ-aminobutyric acid, β-cyano-β-alanine, β-cyanopropionate and α-aminoacetonitrile have K_i values of 0.014, 0.082, 3.2 and 6.2 mM, respectively. The inhibition by each is competitive with glutamic acid. The inhibition by *n*-butyronitrile is non-competitive with the substrate but is reversible with pyridoxal 5'-phosphate. The structurally related L-isoglutamine, glutaric and succinic acids are moderately inhibitory. Administered to chicks at a dosage near 12 mg/100 g, *n*-butyronitrile is sedative. At a dosage of 8 mg, γ-cyano-γ-aminobutyric acid is convulsant and lethal.

INTRODUCTION

Following the early observation that pyridoxal moderates the toxicity of β-cyanoalanine for the rat[1,2], enzymic reactions that require pyridoxal 5'-phosphate (pyridoxal-*P*) have been of special interest in studying the mechanism of action of this neurolathyrogen. One such reaction is mammalian cystathionase which was found to be inhibited strongly by β-cyanoalanine[3]. Another pyridoxal-*P*-requiring reaction that has seemed relevant in this connection is glutamate decarboxylase (L-glutamate 1-carboxy-lyase, EC 4.1.1.15) (refs. 4, 5).

This enzyme is present in the mammal chiefly in nervous tissue, and its regulation is considered to afford a means of modulating neuronal activity *via* the action of the decarboxylation product γ-aminobutyric acid[6]. An investigation of the effect of cyanoamino acids on glutamate decarboxylase from *Escherichia coli* is the subject of the present report. Studied in addition to β-cyanoalanine, the neurotoxin from common vetch[7], have been γ-cyano-α-aminobutyric acid, isolated recently from *Chromobacterium violaceum* cultures[8], γ-cyano-γ-aminobutyric acid (I), β-cyano-β-

alanine (II), and α-cyanoglycine (III), three new synthetic amino acids having the



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unique α-cyanoamino acid structure[9], and a number of related nitriles of simpler structure including β-cyanopropionate, *n*-butyronitrile and α-aminoacetonitrile, an osteolathyrogen[10]. The possible contribution to the inhibition by any inorganic cyanide present in the enzymic digests also has been assessed; in most cases it was small. Also described are pharmacological effects of *n*-butyronitrile and γ-cyano-γ-aminobutyric acid in chicks. γ-Cyanobutyrate required for this study was isolated as an intermediate in a known synthesis of glutaric acid and glutarimide from γ-butyrolactone and cyanide[11].

MATERIALS AND METHODS

E. coli glutamate decarboxylase, Type II purified powder, Preparation A with initial specific activity of 0.8 unit/mg, was purchased from Sigma Chemical Co. It has K_m of 2 mM. Activity was unchanged upon addition of 0.03–2.0 mM pyridoxal-*P*. Preparation B from the same source was maximally activated by 64% by 0.5–2 mM pyridoxal-*P*, when its specific activity was 2 units/mg, and K_m 2.1 mM.

L-[1-^{14}C]Glutamic acid (20 mC/mmole) was purchased from Calbiochem; non-isotopic L-glutamic acid, L-glutamine, L-norvaline, and L-ornithine from Mann Research Laboratories. Pyridoxal-*P* was obtained from both Sigma Chemical Co. and Mann Research Laboratories; cyanoacetamide, cyanoacetic acid, hydracrylonitrile, β,β-iminodipropionitrile, succinonitrile, and nicotinonitrile from Matheson Coleman and Bell; *n*-butyronitrile, propionitrile, glutaronitrile, glutaric acid, β-aminopropionitrile–HCl, succinamic acid, L-α-aminobutyric acid, and mimosine from Aldrich Chemical Co.; 3-butenenitrile and *n*-hexanenitrile from Eastman Organic Chemicals; *n*-valeronitrile from K and K Laboratories; and sodium succinate from Fisher Scientific Co. L-γ-Cyano-γ-aminobutyric acid[9], L-β-cyano-β-alanine[9], α-cyanoglycine[9], sodium β-cyanopropionate hydrate[12], L-β-cyanoalanine[2], L-γ-cyano-α-aminobutyric acid[8], isoasparagine and isoglutamine[13] were synthesized as described elsewhere. α-Aminoacetonitrile was obtained from the bisulfate (Aldrich) and purified by distillation, after which it traveled on paper electrophoresis at pH 5.7 as a single, basic, yellow ninhydrin-positive spot.

Cyanide was determined according to EPSTEIN[14]. Elemental analysis, infrared spectrum, and paper electrophoresis were carried out as described elsewhere[8].

For effects *in vivo* compounds were injected subcutaneously into 6 White Leghorn chicks, 1–3 days old.

Synthesis of sodium γ-cyanobutyrate

The crude reaction product, 3 g, of the condensation of γ-butyrolactone and KCN (ref. 11) was dissolved in 5 ml of water. The solution was acidified in the cold to pH 2 with 6 M HCl and extracted with 70 ml of ether. The extract was washed with water and dried ($MgSO_4$). Evaporation of the ether left a pale yellow liquid. This was taken up in water and titrated to pH 7 with 7 ml of 2 M NaOH. The solution was concentrated in a vacuum.

To the residue were added 10 ml of ethanol followed by excess ether. The gelatinous product was titrurated several times with ether and then collected and dried in air; wt. 1.73 g. Of this material 1.37 g were dissolved in 18 ml of ethanol (water bath). The solution was clarified by centrifugation and then allowed to stand at room temperature overnight. The solid was collected and recrystallized in the same manner, 0.51 g, mp 146–151°. The mother liquors yielded 0.61 g with the same mp. For analysis the material was recrystallized again. Found: C, 43.8; H, 4.42; N, 9.96. $C_5H_6NO_2Na$ requires C, 44.5; H, 4.48; N, 10.4%.

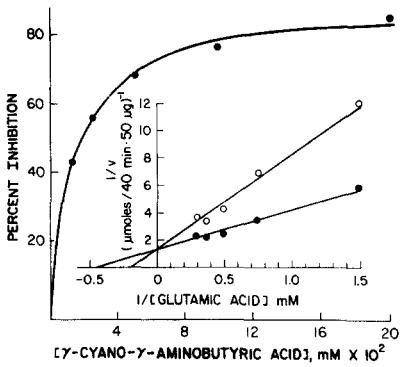
The infrared spectrum (KBr disk) had a sharp absorption peak of moderate intensity at 4.4 μm for the cyano group and strong peaks at 6.3–6.5 μm and 6.9–7.1 μm for the ionized carboxylate group.

Enzyme assays

Reactions were carried out in duplicate in stoppered micro Warburg vessels in 1 ml of 0.1 M sodium acetate buffer, pH 5.0 (ref. 15), with 2 mM L-[1-^{14}C]glutamic acid. Reactions were initiated by addition of 100 μl containing 50 μg of enzyme from the side arm. Liberated $^{14}CO_2$ was absorbed in 1 ml of 1 M NaOH in the inner well. After 40 min, the reactions were terminated by tipping in from the side arm 200 μl of 3 M H_2SO_4. Mixtures were allowed to stand for 60–90 min with occasional swirling. Radioactivity in NaOH was determined on a 100 μl sample in 0.5 ml of Nuclear Chicago solubilizer and 15 ml of BRAY's[16] mixture. After 3 h the solutions were counted in a Nuclear-Chicago 722 liquid scintillation system. In later experiments (K_i determinations of β-cyano-β-alanine, α-aminoacetonitrile, β-cyanopropionate, and *n*-butyronitrile) the procedure was modified with an increase in precision and sensitivity. The solution of 20–100 μg of enzyme was added by pipette. The vessel was stoppered and incubated for 20 min at 37°, and the reaction was terminated after 20 min by the addition of 300 μl of 3 M H_2SO_4 from the side arm. The CO_2 was absorbed onto a 12 mm × 18 mm strip of Whatman No. 3 MM paper wet with 50 μl of 1 M NaOH (ref. 17) and suspended by means of a metal loop clip held in a slit in the rubber stopper. After 90 min at 37° the strips were dried in a stream of warm air and then counted directly in Liquifluor (New England Nuclear) diluted with toluene. In experiments with $Na^{14}CO_3$ recovery of CO_2 was 96 ± 5%.

Approx. 15% hydrolysis took place in the uninhibited reaction, the rate of which was linear with time and quantity of enzyme. Counts/min in CO_2 of the uninhibited reaction *minus* that of the inhibited reaction divided by that of the uninhibited reaction and multiplied by 100 gave the percentage inhibition. Potency was determined by assay with 20 mM glutamic acid or was derived from the $1/v$ intercept of the uninhibited reaction in a Lineweaver–Burk plot.

Enzymic reactions to be analyzed for cyanide were carried out in the outer well of 33 mm microdiffusion vessels and were terminated with 300 μl of H_2SO_4.



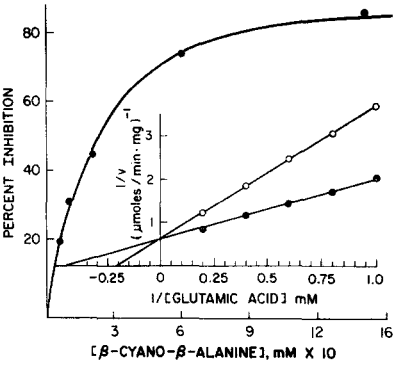
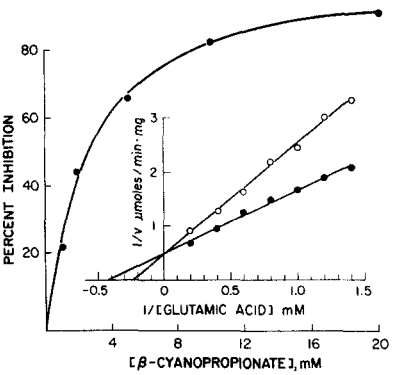


Fig. 1. Kinetics of inhibition of glutamate decarboxylase by γ-cyano-γ-aminobutyric acid. Outer curve: inhibition of the decarboxylation of L-[1-^{14}C]glutamic acid, 2 mM, with increasing amounts of γ-cyano-γ-aminobutyric acid, 12.5–200 μM. Insert: Lineweaver–Burk diagram of the velocity of the decarboxylation of L-glutamic acid (●—●). The upper curve (○—○) shows the velocity of the reaction inhibited by 18 μM γ-cyano-γ-aminobutyric acid (see Table II). *E. coli* enzyme, Preparation A, 50 μg, was used.

Fig. 2. Kinetics of inhibition of glutamate decarboxylase by β-cyano-β-alanine. Outer curve: inhibition of the decarboxylation of L-[1-^{14}C]glutamic acid, 2 mM, with increasing amounts of β-cyano-β-alanine, 12 μM–1.4 mM. *E. coli* enzyme, Preparation A, 50 μg, was used. Insert: Lineweaver–Burk diagram of the velocity of the decarboxylation of L-glutamic acid (●—●). The upper curve (○—○) shows the velocity of the reaction inhibited by 0.15 mM β-cyano-β-alanine (see Table II). *E. coli* enzyme, Preparation B, 20 μg, and 2 mM pyridoxal-*P* were used.



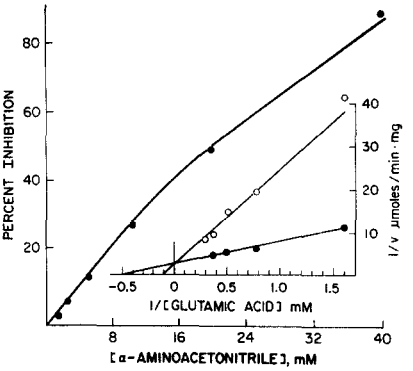


Fig. 3. Kinetics of inhibition of glutamate decarboxylase by sodium β-cyanopropionate. Outer curve: inhibition of the decarboxylation of L-[1-^{14}C]glutamic acid, 2 mM, with increasing amounts of sodium β-cyanopropionate, 1–20 mM. Insert: Lineweaver–Burk diagram of the velocity of the decarboxylation of L-glutamic acid (●—●). The upper curve (○—○) shows the velocity of the reaction inhibited by 2.5 mM sodium β-cyanopropionate (see Table II). *E. coli* enzyme, Preparation B, 20 μg, and 2 mM pyridoxal-*P* were used.

Fig. 4. Kinetics of inhibition of glutamate decarboxylase by α-aminoacetonitrile. Outer curve: inhibition of the decarboxylation of L-[1-^{14}C]glutamic acid, 2 mM, with increasing amounts of aminoacetonitrile, 0.63–40 mM. Insert: Lineweaver–Burk diagram of the velocity of the decarboxylation of L-glutamic acid (●—●). The upper curve (○—○) shows the velocity of the reaction inhibited by 20 mM aminoacetonitrile (see Table II). *E. coli* enzyme, Preparation A, 100 μg, and 1 mM EDTA were used.

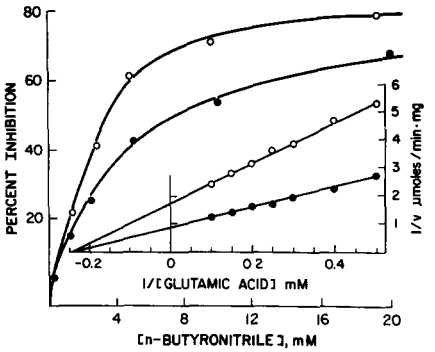


Fig. 5. Kinetics of inhibition of glutamate decarboxylase by *n*-butyronitrile. Outer curves: inhibition of the decarboxylation of L-[1-^{14}C]glutamic acid, 2 mM, with increasing amounts of *n*-butyronitrile, 0.3–20 mM; ○—○, with *E. coli* enzyme Preparation A, 50 μg; ●—●, with enzyme Preparation B, 40 μg, and no added pyridoxal-*P*. Insert: Lineweaver–Burk diagram of the velocity of the decarboxylation of L-glutamic acid (●—●). The upper curve (○— ○) shows the velocity of the reaction inhibited by 8 mM *n*-butyronitrile (see Table II). Enzyme Preparation B, 50 μg, was used with no added pyridoxal-*P*.

Liberated cyanide was absorbed in the inner well by 200 μl of 1 M NaOH. After 3 h this solution was neutralized with 1 N HCl, diluted to 1 ml, and analyzed for cyanide. In experiments with 300 μl of 1 mM KCN recovery of cyanide was 94%.

Inhibition of L-*glutamate decarboxylase*

Tested were 33 compounds including 5 cyanoamino acids and simpler nitriles and other compounds structurally related to the active cyanoamino acids, some lacking one or two of the functional groups. With L-glutamic acid at K_m (2 mM), the substances were added at 10 or 20 mM. Other conditions are given under *Enzyme assays*. Compounds inhibiting the enzyme by 50% or more were tested usually at 5 or 6 other concentrations, and curves were plotted relating concentration of inhibitor to percentage inhibition. For γ-cyano-γ-aminobutyric acid, β-cyano-β-alanine, sodium β-cyanopropionate, α-aminoacetonitrile, and *n*-butyronitrile these plots are shown as the outer curves of Figs. 1–5. From these curves were obtained I_{50}, the concentration of additive inhibiting by 50%, which values are given in Table I along with the percentage inhibition at 10 or 20 mM.

Estimation of the contribution of cyanide to the inhibition by cyanoamino acids and other nitriles

Like glutamate decarboxylase from *Clostridium welchii*[18], the *E. coli* decarboxylase is inhibited by inorganic cyanide. Its I_{50} is 0.02 mM (Table I), a value that is near the I_{50} of γ-cyano-γ-aminobutyric acid. Enzymic digests containing inhibitory α-cyanoamino acids or related nitriles were therefore examined closely for content of inorganic cyanide, especially since α-cyanoamino acids tend to decompose in aqueous solution[9]. Based on 50% inhibition by 0.02 mM cyanide, the percentage inhibition due to cyanide present at the end of incubation was calculated for the concentration of nitrile inhibiting by 50% as

$$\frac{\text{cyanide}}{\text{nitrile}} \times \text{nitrile } I_{50}\ (\text{mM}) \times \frac{50}{0.02}$$

These values are given as the outer column of Table I.

TABLE I

INHIBITION OF *E. coli* GLUTAMATE DECARBOXYLASE BY α-CYANOAMINO ACIDS AND RELATED COMPOUNDS

I_{50} is the concentration of compound inhibiting 20 to 100 μg of enzyme by 50% at 2 mM glutamic acid. Conditions are given under *Enzyme assays*. Unless indicated otherwise enzyme Preparation A was used. Essentially inactive at 20 mM were 2-cyanoacetamide, β-aminopropionitrile–HCl, succinonitrile, sodium γ-cyanobutyrate, L-asparagine, norvaline, α-aminobutyric acid, ornithine, mimosine (2 mM), *n*-hexanenitrile*,**, β-alanine*,**, hydracrylonitrile*,**, and L-glutamine**. Bracketed are % inhibition values corrected for inhibition due to cyanide. For dashes see RESULTS, *Contribution of cyanide to the inhibition by nitriles*.

Compound	*Inhibition at 20 mM (%)*	*I_{50} (mM)*	*Inhibition due to cyanide at end of incubation** (% at I_{50})*
L-γ-Cyano-γ-aminobutyric acid, 12.5 μM–0.2 mM	100 (10 mM)	0.022	1.8
L-β-Cyano-β-alanine, 12.5 μM–1.4 mM	99 (10 mM)	0.23	11.5
α-Cyanoglycine, 0.4–20 mM	89	0.8	4
α-Aminoacetonitrile, 0.63–40 mM (+ 1 mM EDTA)	49	20	5
Sodium β-cyanopropionate, 1–40 mM	89	2.5, 2.5*	—
Cyanoacetic acid	31		—
L-β-Cyanoalanine	22		—
L-γ-Cyano-α-aminobutyric acid	24 [14]		
Glutaronitrile	27 [25]		
Glutaric acid, 0.63–20 mM	63	11	
Succinic acid	47**, 25*		
Succinamic acid	7**, 19*		
n-Valeronitrile	35**, 21*		—
n-Butyronitrile, 0.3–20 mM	77, 65**	3.5, 8**	—
3-Butenenitrile**	9		
Propionitrile	24		—
Acetonitrile	11		
Isoglutamine	41**, 58*, 19		
Isoasparagine	20**		
KCN	100 98.5 (2 mM)	0.02	

* Enzyme Preparation B with 2 mM pyridoxal-*P*.
** Enzyme Preparation B.

Kinetic studies

Lineweaver–Burk plots of the decarboxylation of glutamic acid were made in the absence and presence of γ-cyano-γ-aminobutyric acid, β-cyano-β-alanine, sodium β-cyanopropionate, α-aminoacetonitrile and *n*-butyronitrile and are shown as the inserts of Figs. 1 to 5. Inhibitory compounds generally were used at a concentration giving 40–50% inhibition with 2 mM substrate. Specific conditions and K_i for each inhibitor are given in Table II. The table also notes the change in inhibition with

TABLE II

INHIBITION CONSTANTS OF α-CYANOAMINO ACIDS AND RELATED NITRILES FOR *E. coli* GLUTAMATE DECARBOXYLASE

With *n*-butyronitrile in water enzyme Preparation B was used; with β-cyanopropionate and β-cyano-β-alanine at 1–5 mM Glu, the same preparation and 2 mM pyridoxal-*P*; all others, enzyme Preparation A. Experiments with α-aminoacetonitrile and *n*-butyronitrile in 0.1% ethanol included 1 mM EDTA.

Cyano compound	L-*Glutamic acid (mM)*	*Range of inhibition (%)*	K_i
L-γ-Cyano-γ-aminobutyric acid, 18 μM	0.67–4	25–51	14 μM
L-β-Cyano-β-alanine, 150 μM	0.67–4		82 μM
	1–5	29–45	150 μM
Sodium β-cyanopropionate, 2.5 mM	0.72–5	24–39	3.2 mM
α-Aminoacetonitrile, 20 mM	0.6–3.4	51–76	6.2 mM
n-Butyronitrile, 8 mM, water	2–10	50 ± 1	8.3 mM
2 mM, 0.1% ethanol	0.6–2.7	39 ± 2	2.8 mM
2 mM, 2% ethanol	0.6–2.7	21 ± 2.5	9.1 mM

glutamic acid, which varied from 0.6 to 5 mM. For the competitive reactions K_i was calculated from the intercept on the $1/[S]$ axis of the inhibited and uninhibited reactions, according to

$$\frac{[I]}{K_i} = \frac{\text{inhibitor-free intercept}}{\text{inhibitor intercept}} - 1$$

For the noncompetitive reaction K_i was calculated from the intercept on the $1/v$ axis according to

$$\frac{[I]}{K_i} = \frac{\text{inhibitor intercept}}{\text{inhibitor-free intercept}} - 1$$

RESULTS

Of the nitriles tested γ-cyano-γ-aminobutyric acid, with $K_i = 14\ \mu M$, was the most effective inhibitor. β-Cyano-β-alanine had $K_i = 82\ \mu M$ with enzyme Preparation A and 150 μM with Preparation B and 2 mM pyridoxal-*P*. β-Cyanopropionate and α-aminoacetonitrile had $K_i = 3.2$ and 6.2 mM, respectively. The curves relating inhibitor concentration and percentage inhibition were all hyperbolic in shape, except for α-aminoacetonitrile when inhibiting by more than 50%. As shown in the Lineweaver–Burk plots, γ-cyano-γ-aminobutyric acid, β-cyano-β-alanine, β-cyanopropionate, and α-aminoacetonitrile each acted competitively towards the substrate, L-glutamic acid.

By contrast, *n*-butyronitrile inhibited non-competitively. Over the range of 2 to 10 mM glutamic acid, inhibition by 9 mM *n*-butyronitrile remained at 50%.

However, the inhibition depended on the enzyme preparation (see MATERIALS AND METHODS) and the presence of added pyridoxal-*P* (see Fig. 5 for inhibition curves with enzyme Preparations A and B, and see *Effect of pyridoxal-5'-P on inhibition by nitriles*). Determined with enzyme Preparation B in the absence of added pyridoxal-*P* when $K_m = 4.1$ mM, K_i of *n*-butyronitrile is 8.3 mM. With enzyme Preparation A of $K_m = 2$ mM, *n*-butyronitrile in 0.1% ethanol had $K_i = 2.8$ mM; with 2% ethanol, $K_i = 9.1$ mM; ethanol seems to decrease the affinity of the enzyme for this nitrile. Preincubation with this inhibitor had no effect on the degree of inhibition; when enzyme Preparation A was preincubated with 1 mM *n*-butyronitrile for 0, 20 and 30 min, inhibition remained at 28 ± 2%. The activity of the uninhibited enzyme declined by 7 and 11%.

Effect of pyridoxal-5'-P on the inhibition of glutamate decarboxylase by various nitriles

On addition of 2 mM pyridoxal-*P*, the inhibition of enzyme Preparation B by 0.15 mM β-cyano-β-alanine increased from 24 to 44%; by 3 mM α-cyanoglycine, from 38 to 88%, an effect that is attributed to cyanide formation catalyzed non-enzymically by the added pyridoxal-*P*. There was no effect of 2 mM pyridoxal-*P* on the inhibition by 22 μM γ-cyano-γ-aminobutyric acid and by 2.6 mM β-cyanopropionate,

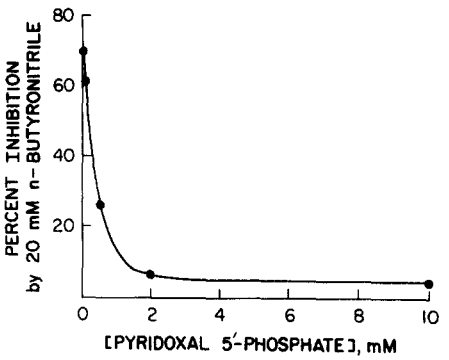


Fig. 6. Effect of pyridoxal-*P* on the inhibition of glutamate decarboxylase by *n*-butyronitrile. Reaction mixtures contained 2 mM L-[1-^{14}C]glutamic acid, 20 mM *n*-butyronitrile, *E. coli* enzyme Preparation B, 40 μg, and increasing amounts of added pyridoxal-*P*, 0.05–10 mM.

which remained at 51 and 55%, respectively. Inhibition by 20 mM *n*-butyronitrile fell from 65 to 5–11%, and it remained essentially the same with 0.5 to 20 mM *n*-butyronitrile. Fig. 6 shows the striking reversal with increasing amounts of pyridoxal-*P* of the inhibitory effect of 20 mM *n*-butyronitrile on glutamate decarboxylase activity.

Contribution of cyanide to the inhibition by nitriles

γ-Cyano-γ-aminobutyric acid and β-cyano-β-alanine gave cyanide to the extent of 3 and 2%, repectively. This corresponded to less than 2 of the 50% inhibition observed with γ-cyano-γ-aminobutyric acid and to 11.5% of that with β-cyano-β-alanine. Of 50% inhibition by 0.8 mM α-cyanoglycine and 20 mM α-aminoacetonitrile, 4 and 5% could be attributed to cyanide; of 24% inhibition by 20 mM γ-cyano-α-aminobutyric acid, 10% to cyanide. Cyanide formation from these was only 0.02–0.2%. No cyanide was detected in the digests of 2.5 mM sodium β-cyanopropionate,

3.5 mM *n*-butyronitrile, and 20 mM propionitrile, *n*-valeronitrile, cyanoacetic acid, and β-cyanoalanine. Any inhibition resulting from cyanide formed from these and present below the limit of detection (0.002 μmole) would be less than 5%.

Effects in chicks

n-Butyronitrile, the non-competitive inhibitor, at dosages of 12 to 27 mg/100 g of body weight, caused long-acting sedation and prostation. L-γ-Cyano-γ-aminobutyric acid, representing the nitriles that inhibit competitively, at dosages of 8 to 19 mg/100 g caused convulsions, depression, and some head retraction followed by death. Both compounds were ineffective at 6 mg/100 g.

DISCUSSION

The finding that γ-cyano-γ-aminobutyric acid, β-cyano-β-alanine, and α-cyanoglycine effectively inhibited glutamate decarboxylase suggests that for high inhibitory activity the three functional groups, cyano, carboxyl, and amino, are desirable. Since the structural isomers γ-cyano-α-aminobutyric acid and β-cyano-alanine were not very active, the α-cyanoamino grouping $\begin{array}{l}\diagdown\\ \text{CHC}\equiv\text{N}\\ |\\ \text{NH}_2\end{array}$ appears to be relevant to the inhibition. α-Aminoacetonitrile had special interest as an inhibitor of glutamate decarboxylase in view of its other known effects as a teratogen[19] and as an inhibitor of collagen maturation[10]. β-Aminopropionitrile, another osteolathyrogen, was not inhibitory. These observations support the suggestion that the α-cyanoamino structure is pertinent to the inhibition of glutamate decarboxylase. Also consistent with this was the finding that isoglutamine and isoasparagine having the α-amino-carboxamide grouping $\begin{array}{l}\diagdown\\ \text{CHCONH}_2\\ |\\ \text{NH}_2\end{array}$ were more inhibitory that the corresponding ω-aminocarboxamides glutamine and asparagine. From the foregoing it would seem that the α-carboxyl of glutamic acid is much more replaceable by the cyano group than is the γ-carboxyl. In the presence of the α-amino and the γ- or β-carboxyl groups, cyano is much more inhibitory than carboxamide. Furthermore, in the presence of the α-cyanoamino group, the ω-carboxyl group is not essential for inhibitory activity.

In the absence of the amino group inhibitory activity was still present, but it decreased. Thus I_{50} for β-cyanopropionate was 10 times that for β-cyano-β-alanine. Likewise, cyanoacetic acid was about one-third as effective as α-cyanoglycine at 20 mM. Thus, in the presence of the cyano and carboxyl groups, the amino group is not required for inhibitory activity. Finally, the finding that *n*-butyronitrile was inhibitory confirmed that the ω-carboxyl- and the α-amino groups are unnecessary and showed that both could be lacking at the same time without losing such activity.

The order of effectiveness of the α-cyanoamino acids as inhibitors was γ-cyano-γ-aminobutyric acid > β-cyano-β-alanine > α-cyanoglycine. Since γ-cyano-γ-aminobutyric acid is closest in size to glutamic acid, the order is in accord with the results

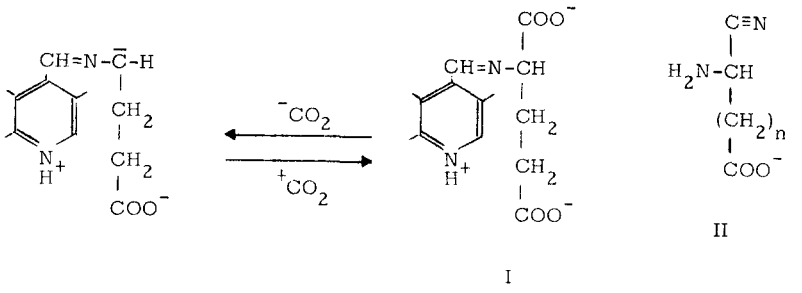


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Fig. 7. Summary of the decarboxylation of L-glutamic acid by glutamate decarboxylase according to WESTHEIMER[20]. α-Cyanoamino acids (II) may be inhibiting the complex (I).

of the kinetic experiments indicating the α-cyanoamino acids to compete with the substrate for the active site of the enzyme. Presumably the cyano group replaces the α-carboxyl group, and the α-amino and ω-carboxyl correspond to these same groups in glutamic acid. In Fig. 7 the coenzyme–substrate complex is shown as I, and the α-cyanoamino acids, as II.

Of five inhibitory nitriles examined, *n*-butyronitrile was the only one whose effect could not be reversed by glutamic acid. As an aliphatic nitrile it showed structural specificity, the order of activity being *n*-butyronitrile > *n*-valeronitrile > propionitrile > acetonitrile > *n*-hexanenitrile > 3-butenenitrile. The cyano group would seem to be a likely point of attachment to the enzyme, and the size and configuration of the side chain also are important. Although the inhibition produced by *n*-butyronitrile was almost completely reversible by pyridoxal-*P*, no indication has been obtained of a direct interaction of pyridoxal-*P* and *n*-butyronitrile, as judged by the lack of effect of 25 mM *n*-butyronitrile on the spectrum of 10^{-5} M pyridoxal-*P* between 290 and 500 mμ at pH 5 and 7. Studies are planned to establish whether the inhibition by *n*-butyronitrile involves removal or separation of pyridoxal-*P* from the enzyme. Whether the interesting pharmacological effects of *n*-butyronitrile and γ-cyano-γ-aminobutyric acid are based on inhibition of glutamate decarboxylase also remains to be determined.

Several dicarboxylic acids were moderately inhibitory. Glutaric acid, known to inhibit the decarboxylase from the plant *Cucurbita moschata*[21], was more active than succinic acid, and each of them was more so than the corresponding dinitrile glutaronitrile and succinonitrile. The inhibitors may belong to the large non-specific group of carboxylic acids that inhibit the plant decarboxylase presumably by competing with the γ-carboxyl group of glutamic acid[21].

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