Enzymatic Decarboxylation of γ-Hydroxyglutamic Acid to α-Hydroxy-γ-amino-*n*-butyric Acid

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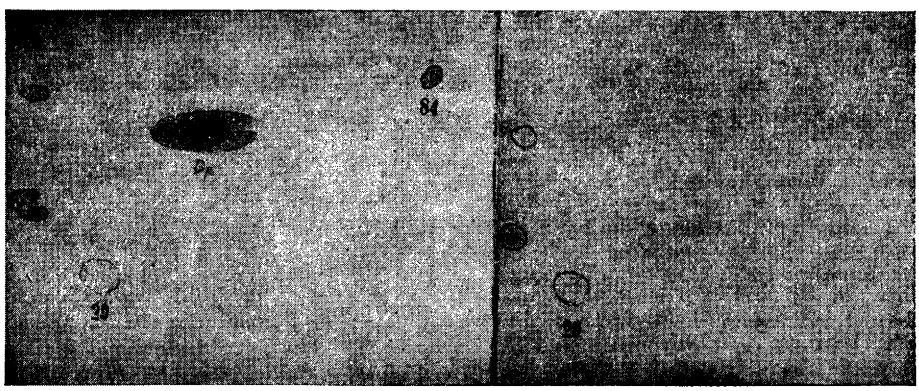
We have recently isolated γ-hydroxyglutamic acid from *Phlox decussata* [1]. Attempts to decarboxylate this acid with homogenates of *Phlox* led to negative results, whereas L-glutamic acid was easily decarboxylated with the same homogenates. Accordingly, *Phlox* did not contain the specific γ-hydroxyglutamic acid decarboxylase. The same result was obtained with homogenates of pea.

After fruitless experiments with green plants we found decarboxylase activity also with γ-hydroxyglutamic acid by using as enzyme material *Escherichia coli*, a B_{12}-vitamin requiring mutant. α-Hydroxy-γ-aminobutyric acid was hereby formed as the decarboxylation product (Fig. 1) which could be isolated and characterized (*cf.* below). This amino acid could not be detected in *E. coli* without addition of γ-hydroxyglutamic acid (70 % alcohol extract of bacteria, two-dimensional paper chromatography).

According to Umbreit and Heneage [2] *E. coli* decarboxylates *allo*-β-hydroxy-DL-glutamic acid (synth.). All strains of *E. coli* capable of decarboxylating this acid contained glutamic acid decarboxylase too, but the results suggested that the two enzymes are different. We have compared the velocities of the decarboxylation of L-glutamic acid, our γ-hydroxyglutamic acid, *allo*-β-hydroxy-DL-glutamic acid and β-hydroxy-DL-glutamic acid using *E. coli*. The CO_2 evolution was measured in a Warburg apparatus in N_2-atmosphere according to Schales *et al.*[3]. The results are presented in Table 1.

Isolation of the decarboxylation product. 27 mg of γ-hydroxyglutamic acid in 10 ml of 0.1 *M* acetate buffer (pH 4.9) + 1 g of fresh *E. coli* (178 mg dry subst.) was kept for 24 h at 38° C with occasional shaking. The bacteria were separated and washed with the buffer. The solution + washwater was passed through an Amberlite IR-120 column. The amino acids remained in the column and were eluted with 1 *N* ammonia. After evaporation *in vacuo*



a b

Fig. 1a. Two-dimensional paper chromatogram (butanol-acetic acid and phenol-NH_3) of decarboxylation of α-amino-γ-hydroxyglutamic acid (84) by E. coli. Dp = decarboxylation product, 84 = traces of not decarboxylated γ-amino-α-hydroxyglutamic acid, 29 = γ-aminobutyric acid.

Fig. 1b. Control experiment without any substrate.

Table 1. Decarboxylation rates of hydroxyglutamic acids. Measurements were made with E. coli at pH 4.9 in a 0.1 M acetate buffer at 37° C and with Phlox and Pisum at pH 6.2 in a 0.1 M phosphate buffer at 30° C with a final substrate concentration of about 5.5 μmoles/ml in each case.

Agent	Q_{CO_2} (μl CO_2/hour/g dry weight)			
	L-Glutamic acid	γ-Hydroxy-glutamic acid	Allo-β-hydroxy-DL-glutamic acid	β-Hydroxy-DL-glutamic acid
E. coli	34 900	3 400	8 200	650
Phlox decussata	730	0		
Pisum sativum	3 000	0		

the solid rest was dissolved in 1 ml of phenol-water-solvent (500:184) and fractionated in a cellulose powder column (1.2 × 37 cm) on the top of which 1 mg methylorange was placed. 17 fractions of 1.5 ml were collected. The indicator came out in fractions 2—5, γ-aminobutyric acid in fractions 6—11, and the decarboxylation product of γ-hydroxyglutamic acid in fractions 11—17. The combined fractions 12—17 (9 ml) were diluted with alcohol and water, the amino acid separated in an Amberlite IR-120 column, eluted with ammonia and evaporated to dryness. After recrystallization from water the mp. was 199° C.

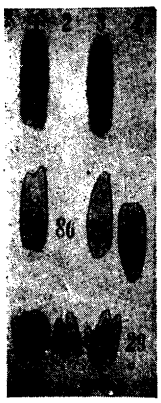


Fig. 2. Paper chromatogram of the pure decarboxylation product after reduction with HI and red P. 1 = reduction products + γ-aminobutyric acid (29), 2 = γ-aminobutyric acid alone, 3 = reduction products, 4 = decarboxylation product alone (86). Solvent phenol-NH_3. The slow moving spots are probably iodine-containing compounds.

Characterization of the decarboxylation product.

1. By reduction with HI (*d* 1.96) and red P at 140° C for 4 h. (1 mg substance + 1 mg P + 6 μl HI) γ-aminobutyric acid was formed (Fig. 2). Accordingly the decarboxylation product was a hydroxyderivative of γ-aminobutyric acid.

2. Analysis: N 11.55. Calc. for a monohydroxyaminobutyric acid N 11.75.

3. The decarboxylation product is not homoserine, and does not contain the α-amino group. Accordingly, the α-carboxyl group is split off by decarboxylation, and the decarboxylation product is α-hydroxy-γ-amino-*n*-butyric acid.

$$HOOC \cdot CHOH \cdot CH_2 \cdot CHNH_2 \cdot COOH \xrightarrow{-CO_2} HOOC \cdot CHOH \cdot CH_2 \cdot CH_2NH_2$$

Transamination: α-ketoglutaric acid + γ-hydroxyglutamic acid ⟶ glutamic acid + γ-hydroxy-α-ketoglutaric acid, could be found with homogenates of *Phlox*.

As far as we know α-hydroxy-γ-amino-*n*-butyric acid has not earlier been found in any biological material.

We are very grateful to Dr. Karl Pfister, Rahway, New Jersey, for preparations of synthetic β-hydroxy-DL-glutamic acid and *allo*-β-hydroxy-DL-glutamic acid.

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Received February 18, 1955.