

OCCURRENCE OF γ -GLUTAMYL- β -CYANOALANINE

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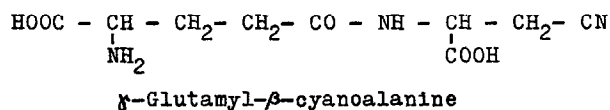
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A study of free amino acids and related compounds in seedlings of Vicia sativa L. and of several other plants in their relation to $H^{14}CN$ assimilation revealed the presence of a strongly labelled ninhydrin-reacting substance of unknown constitution, designed X_1 in preceding experiments^{1,2,3}. This compound is present in higher concentration only in Vicia sativa and near related species. In several other plants examined until now it is detectable only after feeding $H^{14}CN$ to the plants. This unknown compound could be separated from glutamic acid on paper using phenol-water as solvent or by ionophoresis in phosphate buffer pH 7,2. It gave a purple colour with ninhydrin, and behaved as an α -monoamino carboxylic acid when treated successively with cupric nitrate and ninhydrin.

After extracting with 70% ethanol from finely ground seeds of Vicia sativa and partially purifying by ion exchange chromatography⁴, the acidic fraction, containing aspartic acid, glutamic acid, β -cyanoalanine, and the compound X_1 , was separated by ionophoresis. The crude material of X_1 , concentrated at room temperature under reduced pressure, was recrystallized repeatedly from the azeotrop system propan-1-ol-water, m.p. 178° . A sample of 2,0 mg was hydrolyzed in 6 N hydrochloric acid for 24 hours at 105° , and the hydrolysate was used for analysis by paper chromatography and by iono-

phoresis. Only aspartic acid and glutamic acid were found, in a ratio 1:1. Another sample was converted into the DNP-derivative, according the method of Lewy⁵. Free aspartic acid and DNP-glutamic acid were observed after acid hydrolysis. By refluxing 2,0 mg of X₁ with 10 ml 0,1 N NaOH for 3 hours, the compound was converted into a neutral amino acid, showing a high R_F value on paper chromatograms run in phenol-water and in propan-1-ol-water. The DNP-derivative of this compound, isolated by paper chromatography, also yields aspartic acid and DNP-glutamic acid after hydrolysis in 6 N hydrochloric acid. But among the degradation products dinitroaniline was detectable, too. Another sample of X₁ was treated with Sn and HCl. The reaction products were purified by ion exchange chromatography, hydrolyzed in 6 N HCl and analyzed. On the chromatograms and after ionophoretic separation only three spots were observed, corresponding with glutamic acid, aspartic acid, and α,γ -diaminobutyric acid.

The results of the experiments presented here, led us to suggest the following structure for the compound X₁:



These results are in agreement with those of Ressler et al.⁶, who recently isolates the same compound, searching for the neurotoxic factors of Vicia sativa.

In this connection it is remarkable that β -cyanoalanine and γ -glutamyl- β -cyanoalanine are the first detectable H¹⁴CN assimilation products not only in Vicia sativa, but also in all the other plants examined till now^{2,3,7}. It is not yet known, if the biosynthetic pathways, observed after H¹⁴CN feeding to the plants, are occurring in the normal metabolism of these plants, too. Neverthe-

less it has been shown that the radioactive carbon atom of supplied hydrocyanic acid flows via β -cyanoalanine into the metabolism. In Vicia sativa and several other Vicia species β -cyanoalanine as well as γ -glutamyl- β -cyanoalanine were accumulated. Many other plants fed with $H^{14}CN$ accumulate asparagine, synthesized from previously formed β -cyanoalanine, and in $H^{14}CN$ fed Lathyrus odoratus seedlings the Lathyrus factor is one of the especially labelled compounds. Therefore it is possible that this γ -glutamyl- β -aminopropionitrile is synthesized from β -cyanoalanine via γ -glutamyl- β -cyanoalanine by a simple decarboxylation.

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