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METABOLISM OF HYDROCYANIC ACID—III.

ASSIMILATION OF H^{14}CN BY *LATHYRUS ODORATUS* L., *VICIA SATIVA* L., AND *RICINUS COMMUNIS* L.

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Abstract—Radioactive cyanide is quickly assimilated by seedlings of *Lathyrus odoratus* L., *Vicia sativa* L., and *Ricinus communis* L. and β-cyanoalanine and asparagine are the first radioactive compounds produced in all these plants. The biosynthesis of γ-glutamyl-β-aminopropionitrile (Lathyrus factor) and of ricinine are discussed in relation to the H^{14}CN incorporation.

INTRODUCTION

IN ADDITION to cyanogenic glycosides, several other compounds containing a cyano group are known constituents of higher plants. γ-Glutamyl-β-aminopropionitrile (Lathyrus factor) has been isolated from *Lathyrus pusillus* L. and *Lathyrus odoratus* L.,[1,2] the alkaloid ricinine occurs in *Ricinus communis* L., and recently, β-cyanoalanine has been shown to be present in seeds of *Vicia sativa* L.[3] Cyanide is known to be assimilated by higher plants,[4,5] and we have now observed in feeding experiments that the radioactivity from H^{14}CN is incorporated into these compounds.

RESULTS

The radioactivity of ^{14}C-labelled hydrocyanic acid is incorporated strongly into the soluble and the insoluble fraction of 6- to 8-day-old *Lathyrus odoratus* seedlings, after feeding gaseous H^{14}CN in continuous light (Table 1).

The distribution of the radioactivity within the soluble fraction after 48 hr of feeding is shown in Fig. 1, where the ninhydrin-treated two-dimensional chromatogram of a seedling extract is compared with the corresponding autoradiograph. Asparagine (Asp-NH_2), β-cyanoalanine (Cya) and γ-glutamyl-β-aminopropionitrile, the Lathyrus factor (Lat), are the most heavily labelled compounds. Some less intense spots are also observed on the autoradiograph.

Labelled γ-glutamyl-β-aminopropionitrile (containing 3×10^4 cpm/μmole) was isolated by chromatographic methods and hydrolysed with 6 N HCl during which no decrease of radioactivity occurs. The degradation products, glutamic acid and β-alanine, were separated by

[1] H. P. DUPUY and J. G. LEE, *J. Amer. Pharm. Ass.* **43**, 61 (1954).
[2] G. F. MCKAY, J. J. LALICH, E. D. SCHILLING and F. M. STRONG, *Arch. Biochem. Biophys.* **52**, 313 (1954).
[3] CH. RESSLER, *J. Biol. Chem.* **237**, 733 (1962).
[4] B. TSCHIERSCH, *Flora* **153**, 115 (1963).
[5] S. BLUMENTHAL-GOLDSCHMIDT, G. W. BUTLER and E. E. CONN, *Nature, Lond.* **197**, 718 (1963).

paper chromatography in *n*-propanol–water. Most of the radioactivity was present in the β-alanine, and only a little was detected in the glutamic acid. Preliminary degradation experiments with ninhydrin show that most of the radioactivity of β-alanine is localized in the carboxyl group, corresponding to the cyano group of the Lathyrus factor.

Similar feeding experiments were also carried out with *Vicia sativa* seedlings. The results, showing an incorporation of $H^{14}CN$ into these plants, are given in Table 1. Although both *Lathyrus* and *Vicia* assimilate the supplied hydrocyanic acid in the same manner, considerable differences occur in the distribution of the radioactivity. In Fig. 2, a ninhydrin-treated chromatogram of the soluble fraction of *Vicia* seedlings and the corresponding autoradiograph are compared. In these plants only a little radioactivity is localized in the asparagine molecule, but β-cyanoalanine and an unknown compound X_1 are very strongly labelled. High radioactivity was also present in the position of α,γ-diaminobutyric acid (DABS).

TABLE 1. RADIOACTIVITY OF THE 70% ETHANOL SOLUBLE FRACTIONS AND OF THE HYDROLYSED RESIDUES AFTER FEEDING 49·3 μc $H^{14}CN$ ($= 3 \times 10^{-5}$ mole) TO SEEDLINGS OF SEVERAL PLANTS. (cpm/seedling $\times 10^{-3}$)

Plant	Soluble fractions	Insoluble fractions	Total radio-activity
Lathyrus odoratus L.			
48 hr	1444	126	1570
120 hr	4029	202	4231
Vicia sativa L.			
4 hr	70	20	90
48 hr	984	200	1184
72 hr	1732	240	1972
Ricinus communis L.			
48 hr	1080	184	1264

In feeding experiments with $H^{14}CN$ for 4 hr, only β-cyanoalanine and the unknown compound X_1 are labelled, and in short-time experiments a single radioactive spot corresponding with β-cyanoalanine was observed.

In contrast to all the plants examined so far, *Vicia sativa* contains β-cyanoalanine and X_1 in very high concentrations. From seedlings, fed with $H^{14}CN$, β-cyanoalanine and the compound X_1 were isolated by electrophoretic methods. After hydrolysis in 6 N HCl, both β-cyanoalanine and the unknown compound X_1 yield aspartic acid as the only radioactive degradation product. In two feeding experiments, *Vicia* seedlings were supplied with a solution of radioactive β-cyanoalanine and of radioactive X_1. After 6 hr, β-cyanoalanine and the compound X_1 were found to be the compounds most highly labelled in either case.

When *Ricinus communis* seedlings are fed with gaseous $H^{14}CN$, radioactivity is incorporated in large amounts (Table 1). Asparagine is the most highly labelled compound in the soluble fraction. This result is in agreement with the distribution of the radioactivity after cyanide assimilation, observed in numerous plants. Therefore, cyanide will be assimilated by *Ricinus* seedlings also on the pathway via β-cyanoalanine and asparagine, shown to be present in *Linum* and *Vicia*.[6]

[6] B. TSCHIERSCH, *Flora* (in press).

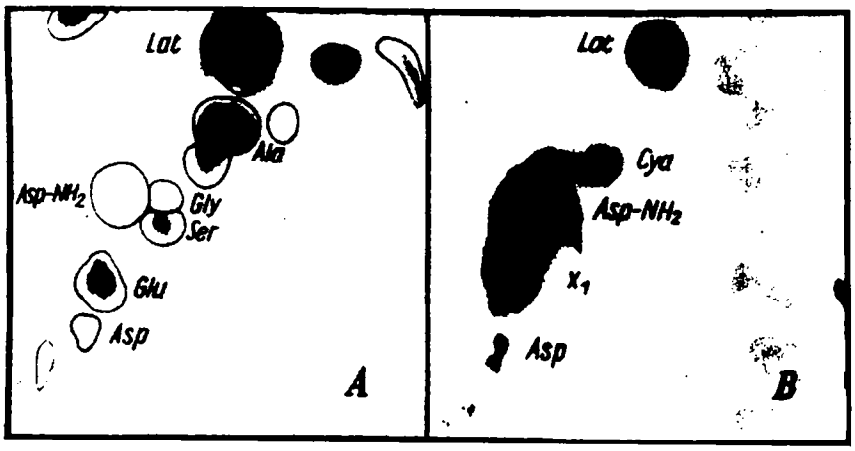

FIG. 1. DISTRIBUTION OF THE RADIOACTIVITY ON TWO-DIMENSIONAL CHROMATOGRAMS OF THE SOLUBLE FRACTION OF *Lathyrus odoratus* L. AFTER $H^{14}CN$ FEEDING.

A. Sprayed with ninhydrin. B. Autoradiograph.

The solvent of the first direction, from the left to the right: propan-1-ol–water (3:1), the second one, from the bottom to the top: phenol–water (4:1).

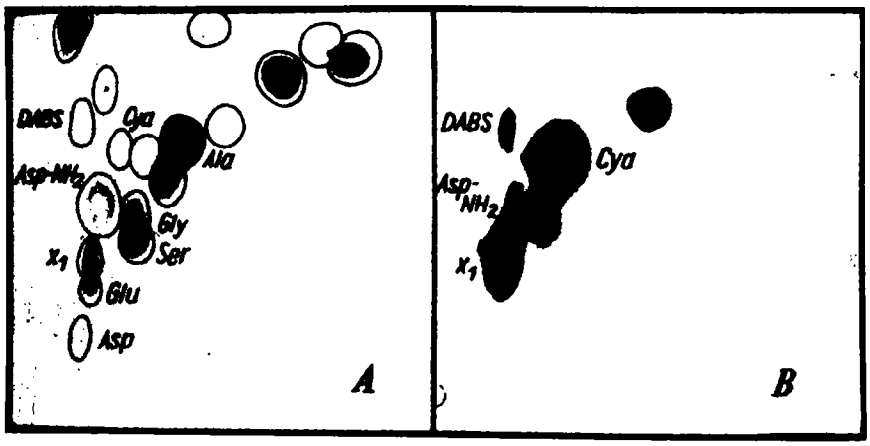

FIG. 2. DISTRIBUTION OF THE RADIOACTIVITY ON TWO-DIMENSIONAL CHROMATOGRAMS OF THE SOLUBLE FRACTION OF *Vicia sativa* L. AFTER $H^{14}CN$ FEEDING.

A. Sprayed with ninhydrin. B. Autoradiograph.

The solvent of the first direction, from the left to the right: propan-1-ol–water (3:1), the second one, from the bottom to the top: phenol–water (4:1).

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DISCUSSION

All plants examined so far are able to assimilate $H^{14}CN$ in the same manner, i.e. by changing a 3-C-compound, probably serine,[6] into β-cyanoalanine. In *Vicia sativa*, β-cyanoalanine is present in higher concentrations, and after feeding $H^{14}CN$, this amino acid is particularly rapidly and heavily labelled. In other plants, β-cyanoalanine is present in lower concentrations, and only detected after feeding radioactive cyanide to the plants. Here asparagine is the compound most heavily labelled. Therefore, we suggest that the radioactivity of the supplied $H^{14}CN$ enters into the metabolism via β-cyanoalanine and asparagine in all the plants.[6]

In *Vicia* seedlings, besides β-cyanoalanine a strongly labelled unknown compound X_1 was observed after $H^{14}CN$ feeding. In other plants, supplied with radioactive cyanide, this compound is also detectable. In feeding experiments and by acid hydrolysis, it has been shown that X_1 is closely related to β-cyanoalanine.

It is remarkable, that in *Vicia* seedlings and in *Lathyrus* seedlings, α,γ-diaminobutyric acid or the Lathyrus factor are products of HCN assimilation and β-cyanoalanine conversion. The experiments with *Lathyrus* seedlings show, that γ-glutamyl-β-aminopropionitrile is mainly labelled in the aminopropionitrile part of the molecule and that the radioactivity is expecially localized in the cyano group. These results led us to consider a relation between Lathyrus factor and β-cyanoalanine or asparagine. β-Cyanoalanine could be utilized in the biosynthesis of the Lathyrus factor, by decarboxylation and formation of the peptide bond. But asparagine may also serve as a precursor, if the carboxamide group was subsequently changed into a cyano group.

Examining the ricinine biosynthesis, a high radioactivity has been observed in the cyano group of the alkaloid after feeding $H^{14}CN$ to the seedlings.[7] The results presented here show that the hydrocyanic acid, supplied to the plants in these experiments, is incorporated into the C-4-atom of aspartic acid. By Mothes *et al.*,[8] it has been shown that aspartic acid-4-^{14}C led to a nicotinic acid only labelled in the carboxyl group, and that this amino acid is the probable precursor for the carbon atoms 2, 3, 8 and the nitrogen atom 1 of the ricinine molecule.[9] In this connexion it is not yet known, whether aspartic acid is the only precursor, or whether ricinine may also be synthesized from asparagine or even from β-cyanoalanine.

Cyanogenic compounds have been found neither in *Ricinus* nor in *Lathyrus*, thus cyanide assimilation probably is without interest in normal metabolism of these plants, but in the metabolism of the 'hydrocyanic acid plant' *Vicia sativa* a formation and assimilation of cyanide may occur. At all events, an indication is given in the results presented here that cyano groups may be synthesized in plants not only by dehydration of carboxamide groups, but also in the course of HCN assimilation.

[7] U. Schiedt and G. Boeckh-Behrens, *Z. physiol. Chem.* **330**, 58 (1962).
[8] E. Mothes, D. Gross, H. R. Schütte and K. Mothes, *Naturwiss.* **48**, 623 (1961).
[9] K. Mothes and H. R. Schütte, *Angew. Chem.* **75**, 265 (1963).