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Short Communications

On Some Unknown Metabolic Intermediates in Plants

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In an earlier paper [1] from this laboratory it was reported that several unknown compounds were rapidly labelled with ^{14}C after infiltration of radioactive pyruvate into the leaves of *Phlox decussata* and *Asplenium septentrionale* (Figs. 1 and 2). When these compounds were subjected to acid hydrolysis (6 N HCl, 20 h, 105°C, sealed tube, except in case of compound No. 11, 1 N HCl, 2 h) the following results were obtained:

Spot No. 5. The only hydrolysis product detected was active alanine.

Spot No. 6. Active glutamic acid and a ninhydrin positive inactive compound, probably identical with glucosamine, were formed. It is likely that this compound may be a derivative of glutamic acid and glucosamine. It is interesting to notice that the amino group of glucosamine-6-phosphate is known to be derived from the amide-group of glutamine [2,3].

Spot No. 7. This is probably identical with compound X in *Asplenium septentrionale*. Both compounds are rapidly and heavily labelled in the beginning of the experiment, the activity decreasing soon thereafter. As the other unknown compounds in question it was first thought to be ninhydrin negative, but in one case a slight brownish-violet color developed after some hours. Therefore it is thought that

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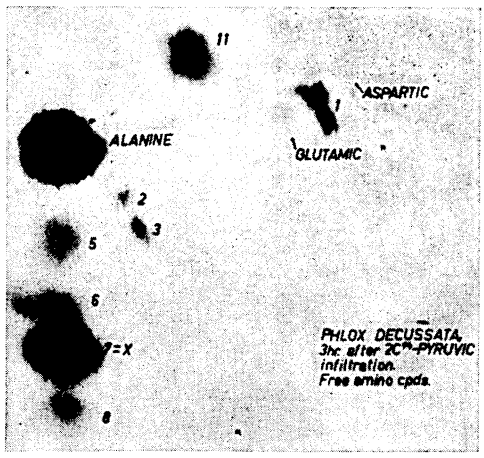

Fig. 1. Radioautogram of free amino compounds 3 h after 2—^{14}C-pyruvate infiltration into *Phlox decussata*.

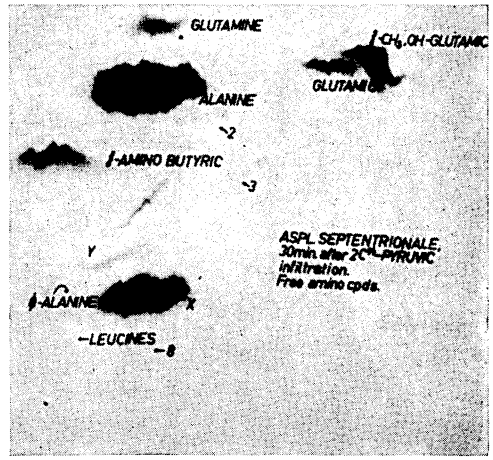

Fig. 2. Radioautogram of free amino compounds 30 min after 2—^{14}C-pyruvate infiltration into *Aspl. septentrionale*.

these compounds in general may be present only at very low concentrations, hence the specific activity being very high. On hydrolysis only one heavily active spot, again ninhydrin negative, was found. This unknown product travelled with the phenol frontier at the level of the leucines in butanol acetic acid solvent. The same result was obtained both on strong and mild hydrolysis.

Spot No. 8. Only spot found on hydrolysis was active alanine.

Spot No. 11. This compound was subjected only to mild hydrolysis and it seemed to be unaffected under these conditions. The spot was, however, most probably superimposed by an inactive spot, because great amounts of inactive γ-hydroxyglutamic acid were formed. The other possibility would be that the compound should have been only partially hydrolysed, the active carbon atom being split off. Such a case, where only one carbon atom becomes labelled after a short time, has been detected during photosynthetic $^{14}CO_2$ assimilation by blue-green algae [4]. In this case only the carbamyl group of the heavily labelled citrulline was found active.

No attempts have so far been made to identify the possible carbohydrates or other ninhydrin negative compounds which

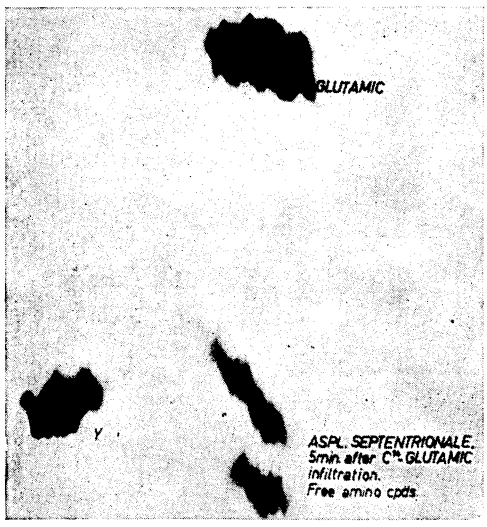

Fig. 3. Radioautogram of free amino compounds 5 min after 1—^{14}C-glutamate infiltration into *Aspl. septentrionale.*

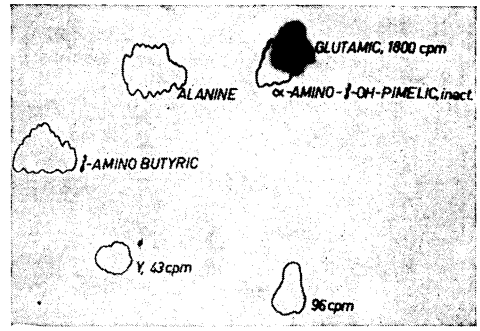

Fig. 4. Radioautogram of the products of hydrolysis of compound Y. Drawn spots are inactive ninhydrin positive compounds. Inactive glutamic acid, alanine and γ-aminobutyric acid were added on the chromatogram.

may arise during the hydrolysis of these unknown compounds.

When 1—^{14}C-glutamic acid was infiltrated into *Asplenium septentrionale* plants only three unknown compounds were found (Fig. 3). On acid hydrolysis of the major spot Y, active glutamic acid was formed in addition to inactive α-amino-γ-hydroxypimelic acid (Fig. 4) which was identified by means of paperchromatography. Even though the nature of this compound is unknown it is of great interest that α-amino-γ-hydroxypimelic acid is found bound to a compound formed as early as during a few minutes. Most of the glutamic acid is evidently readily decarboxylated thus losing its active carbon atom. If the primary reaction would be transamination to γ-ketoglutaric acid, at least some activity would be expected in other amino acids arising *via* the tricarboxylic acid cycle. It was of great interest to notice, too, that no active glutamine was found though glutamine was heavily labelled after 2—^{14}C-pyruvate and slightly labelled even after 1—^{14}C-pyruvate infiltration [1]. This is in good agreement with the assumption that glutamine may arise *via* γ-aminobutyric acid.

It has been known for a few years that the fraction of the 70 % ethanol extract of plant tissues, which passes through a cation exchange resin column, contains bound amino acids liberated on acid hydrolysis [5]. When hydrolysed after 2—^{14}C-pyruvate infiltration into *Asplenium septentrionale* mainly glutamic acid, alanine,

Table 1. The incorporation of activity into the amino acids in the hydrolysates of the "peptide" fraction after 2—^{14}C-pyruvate infiltration into *Aspl. septentrionale*.

Time, h	½	1	2	24
Glutamic, cpm	140	493	266	60
Alanine, cpm	243	175	94	
γ-Aminobutyric, cpm	94	163	—	—
Valine, cpm	15	—	—	—

and α-aminobutyric acid were found labelled in this fraction. Alanine was the major labelled compound after half an hour, but already after one hour the activity of glutamic acid was greater (Table 1). Whereas alanine decreased from the "peptide" fraction it increased in the proteins. However, the activity of compounds 5, 6, 7, 8 and 11 also had a peak within 10 to 30 min when 2—^{14}C-pyruvate alone was infiltrated. Glutamine was not labelled in this case. Inactive glycine, when infiltrated together with active pyruvate, caused a great inhibition of the labelling of these compounds during the first minutes after infiltration, whereafter the activity of compounds 6 and 7 as well as that of glutamic acid greatly increased, showing again the relationship of compound 6 to glutamic acid. In this case even glutamine was slightly labelled after 3 h. Glycine had only a slight inhibitory effect to the alanine containing compounds 5 and 8, while the activity of alanine itself increased due to the greater amount of nitrogen available for transamination reactions.

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The Influence of Anharmonicity upon the Vibrational Probability Density and Mean Amplitudes in Diatomic Molecules

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The influence of anharmonicity upon the vibrational probability density and mean amplitudes in diatomic molecules has been investigated by using two different models to describe the molecular vibrations:

a) an anharmonic oscillator with perturbation terms of first and second order in the potential function,

b) an anharmonic oscillator described by a Morse potential function.

For case a) the probability density $w(r)$, where $r = R - R_e$ is the deviation of the interatomic distance R from its equilibrium value R_e, has been calculated by two different methods. In the first place, an expression for $w(r)$ has been developed by a superposition of probability densities for the different stationary states, according to the equation

$$w(r) = \sum_v |\psi_v(r)|^2 \exp(-E_v/kT) / \sum_v \exp(-E_v/kT)$$

where E_v and ψ_v are the corresponding eigenvalues and normalized eigenfunctions for the vibrational state v, k is Boltzmann's constant, and T is the absolute temperature. The eigenvalues E_v and eigenfunctions ψ_v have been obtained by a second order perturbation calculation similar to that performed by Hutchisson [1] (the results given by this author are, however, somewhat erroneous), and the sums have been evaluated by using well known properties of the Hermite orthogonal functions.

In the second place, the probability density $w(r)$ has been calculated by making use of an analogy to the solution of the time-dependent Schrödinger equation for the system (compare Bloch [2] and Kennard [3]).

In both cases the calculations are somewhat tedious, the result is, however, quite simple:

$$w(r) = (a_0 + a_1 r + a_2 r^2 + a_3 r^3 + a_4 r^4 + a_6 r^6) w^\circ(r)$$