The FORMATION OF ACETIC ACID FROM p-HYDROXYCINNAMIC ACID DURING ITS DEGRADATION TO p-HYDROXYBENZOIC ACID IN WHEAT SHOOTS

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Our investigations on the biosynthesis of methylsalicylate and other benzoic acids in Gaultheria procumbens (Grisebach and Vollmer, 1963; 1964) and independent studies by other workers (El-Basyouni et al., 1964; Ibrahim, 1964; Kindl and Billek, 1964; Zenk and Müller, 1964) have shown that in higher plants benzoic acids can be formed from cinnamic acids by loss of 2 carbon atoms from the side chain. The most reasonable mechanism for this reaction is a ß-oxydation of the cinnamic acid in which case acetic acid would be the other degradation product. However, other mechanisms such as loss of the C_2-fragment as glyoxylic acid, oxalic acid [1], or a stepwise degradation of the side chain must be considered.

El-Basyouni et al. (1964) have shown that in wheat shoots p-coumaric acid-[ß-^{14}C] (p-hydroxycinnamic acid) is degraded to p-hydroxybenzoic acid-[carboxyl-^{14}C]. We have

[1] If oxaly-CoA is formed this could be reduced by glyoxylate dehydrogenase [EC 1.2.1.17] to glyoxylic acid.

now been able to demonstrate that acetic acid-[1-^{14}C] is formed from p-coumaric acid-[carboxyl-^{14}C] in this plant.

An unequivocal proof for the formation of acetic acid from a ^{14}C-labeled precursor during a metabolic process is possible by determining the distribution of radiocarbon in glutamic and aspartic acid (Black and Kleiber, 1957; Reisener et al. 1963). In parallel experiments leaf segments of 7 day old wheat plants were incubated with the labeled compounds shown in table I.

Table I

Uptake of the labeled precursors by the wheat leaves

Precursor	Radioactivity administered (μC)	Radioactivity taken up by the leaves (%)	Radioactivity in respiratory CO_2 (%)
p-coumaric-acid-[1-^{14}C] (I)	8.8	93	0.5
acetic acid-[1-^{14}C] (II)	10.0	88	2.5
oxalic acid-[^{14}C] (III)	10.0	95	1.2
glyoxylic acid-[2-^{14}C] (IV)	10.0	77	1.0
sodium-bicarbonate-[^{14}C] (V)	60.0	100	42.0

10 μmoles of each compound in phosphate buffer (pH 4.7 for I-IV) were fed to 15 g of cut wheat leaves (little club wheat) during 2 hours in complete darkness in a Roux bottle.

After 2 hours the free amino acids were extracted in the usual way and separated on Dowex-1 (Hirs et al., 1954). The glutamic and aspartic acids were diluted with inactive material, purified to constant specific activity and degraded.

Tables II and III and Fig. 1 show the incorporation rates and the distribution of activity in the two amino acids after administration of the various compounds.

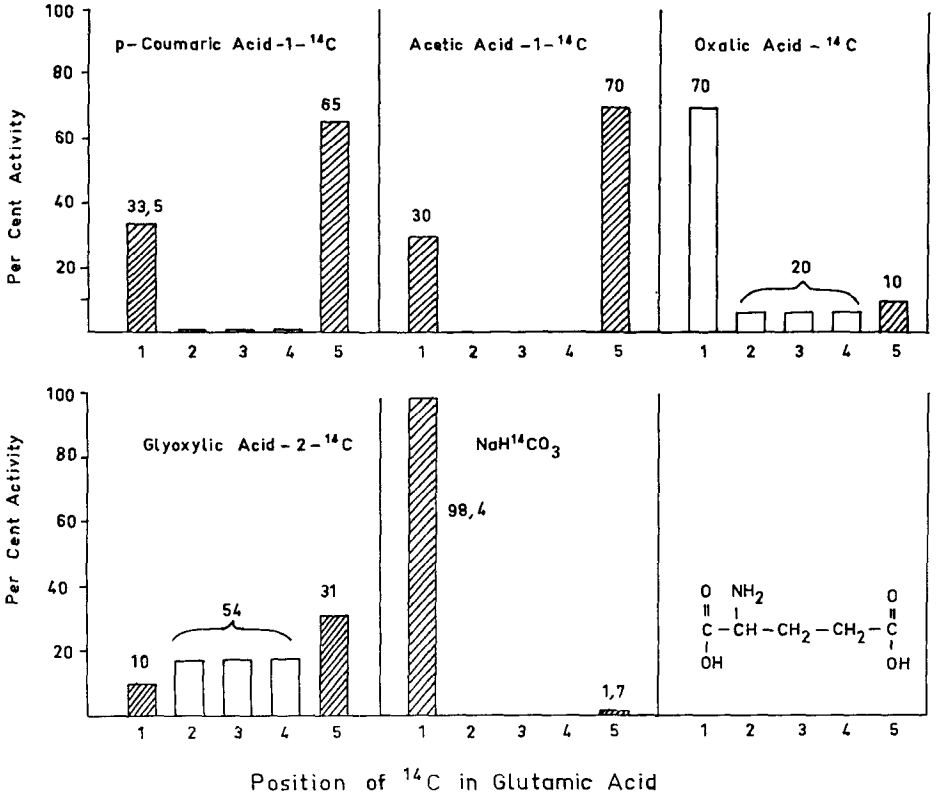

Fig. 1. Distribution of ^{14}C in glutamic acid. Open bars are calculated by difference. Degradations were carried out with ninhydrin (Black and Kleiber, 1957), chloramin T (Mosbach et al., 1951) and hydrazoic acid (Adamson, 1939). The radioactivity in C-1 to C-4 and C-2 to C-5 was determined in each case as a control. Radioactive measurements were made after combustion of the substances to CO_2 according to the method of Simon et al. (1959).

With acetic acid-$[1\text{-}^{14}C]$ as the precursor C-1 of glutamic acid should contain 33,3 per cent and C-5 66,7 per cent of the radioactivity, if the glyoxalate pathway is not impor-

Table II

Incorporation of different precursors into amino acids

Precursor [a]	Glutamic Acid Incorporation Rate (%)	Dilution [b]	Aspartic Acid Incorporation Rate (%)	Dilution
I	0.01	$1,3 \cdot 10^4$	0.008	$1,3 \cdot 10^4$
II	2.5	60	0.9	100
III	0.01	$1,5 \cdot 10^4$	0.014	$5,8 \cdot 10^3$
IV	0.09	$2,4 \cdot 10^3$	0.038	$2,8 \cdot 10^3$
V	0.15	$2,0 \cdot 10^3$	0.1	$1,5 \cdot 10^3$

[a] see Table I [b] $\frac{\text{specific activity of precursor}}{\text{specific activity of procudct}}$

Table III

Distribution of Radioactivity in Aspartic Acid

Precursor [a]	Per cent activity in C-1 + C-4 of Aspartic Acid
I	97
II	98
IV	35
V	93

[a] see Table I Degradations were carried out with ninhydrin.

tant and the total radioactivity in aspartic acid must be in the carboxyl groups (Mc Connell and Finlayson, 1964; Black and Kleiber, 1957; Reisener et al., 1963). It can be seen from Fig. 1 and Table III that the values found correspond almost exactly with the theoretical values when either acetic acid-$\left[1\text{-}^{14}C\right]$ or p-coumaric acid-$\left[\text{carboxyl-}^{14}C\right]$ is the precursor given.

The much lower incorporation rate into the amino acids with p-coumaric acid as precursor was to be expected since the greatest part of the acid is probably trapped in a bound form (El-Basyouni and Towers, 1964) or used for the biosynthesis of lignin (Brown and Neish, 1956) and other phenylpropane derivatives in the plant.

Our results strongly corroborate the assumption that cinnamic acids are degraded in the plant by a mechanism analogous to the ß-oxydation of fatty acids.

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