ON THE FATE OF TYROSINE IN TOBACCO CALLUS TISSUE

II. THE LACK OF INCORPORATION INTO LIGNIN

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[*Manuscript received February* 1, 1962]

Summary

The presence of lignin in an insoluble fraction obtained from tobacco callus tissue was shown by the production of vanillin and syringaldehyde on oxidation of the material with nitrobenzene in alkali.

Tyrosine was not rapidly incorporated into the coniferyl and syringyl residues of the lignin.

The specific activity of the *p*-hydroxybenzaldehyde recovered was higher than that of the bound tyrosine in the material, indicating that a second source of this aldehyde is present. Tyrosine is rapidly incorporated into this second source of *p*-hydroxybenzaldehyde.

I. INTRODUCTION

In a previous paper by Dougall and Shimbayashi (1960) it was reported that tobacco callus tissue yielded, on fractionation, four different nitrogen-containing fractions. One of these, characterized by its insolubility, was called the cell wall residue. Acid hydrolysis of this fraction yielded 18 amino acids which suggested the presence of protein. When the tissue was cultured in the presence of radioactive tyrosine, the cell wall residue became radioactive. Only a small part of the radioactivity of the fraction could be recovered in the tyrosine liberated by acid hydrolysis. Ibrahim, Lawson, and Towers (1961) have also reported similar low recoveries in the tyrosine in their experiments.

Brown and Neish (1956) have shown that tyrosine can act as a precursor of lignin in some plants and Barnoud (1956) has shown that some plant tissues in culture contain lignified elements. It was therefore important to examine the possibility that the low recovery of radioactive tyrosine was due to its incorporation into lignin in the cell wall residue.

The radioactivity of lignin has been assessed by measurement of the radioactivity of vanillin produced by the oxidation with nitrobenzene in alkali. *p*-Hydroxybenzaldehyde may also appear with vanillin on oxidation of plant material. Brown, Wright, and Neish (1959) concluded that this aldehyde was not as reliable as vanillin for the assessment of the radioactivity of lignin when they found that the tyrosine recovered from their plant material had the same specific activity as the *p*-hydroxybenzaldehyde. Although tyrosine bound in plant material will contribute to the *p*-hydroxybenzaldehyde, studies have shown that isolated lignins which do not contain protein will also yield *p*-hydroxybenzaldehyde on nitrobenzene oxidation

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(Bondi and Meyer 1948; de Stevens and Nord 1953). The place of *p*-hydroxybenzaldehyde as a fragment of lignin is therefore established but its isolation indicates the presence of lignin only in the demonstrated absence of bound tyrosine in the sample.

II. Materials and Methods

The cell wall residue used in these experiments was part of the material isolated by Dougall and Shimbayashi (1960).

The material (40 mg) was oxidized with nitrobenzene in alkali by the micromethod of Stone and Blundell (1951). The method of Bland and Stamp (1955) for the isolation of the degradation products of lignin was used in the following modified form. The alkaline solution was extracted three times with one-third its volume of chloroform and then adjusted to a pH between 4·0 and 5·0 with acetic acid. The phenolic compounds were then extracted five times with one-third volume of chloroform and dried over sodium sulphate. The chloroform was evaporated by heating in a water-bath and in an air stream.

The *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde were separated by paper chromatography in the solvent systems of Bland and Stamp (1955) and Bland (1955). No attempt was made to obtain a quantitative recovery. The aldehydes were located by their fluorescence in ultraviolet light and identified by their correspondence with authentic specimens used as markers on the sides of the sheets. Three solvent systems were used. First *p*-hydroxybenzaldehyde was separated from the other aldehydes in benzene–methanol–water (7 : 7 : 10 v/v). The two fractions were eluted separately with methanol and rechromatographed. The *p*-hydroxybenzaldehyde was chromatographed in the system methanol–isopropyl ether–water (1 : 1 : 1 v/v) and the same system was used to separate the vanillin from the syringaldehyde. The three compounds were eluted separately, rechromatographed once in the methanol–isopropyl ether–water solvent, and then twice in benzene saturated with water. The aldehydes were eluted with ethanol, 0·2% ethanolic potassium hydroxide (0·8 ml) added, and the volume adjusted to 10 ml with ethanol. In this solvent *p*-hydroxybenzaldehyde had an absorption maximum at 339 mμ, vanillin at 354 mμ, and syringaldehyde at 372 mμ. At these specific wavelengths standard curves relating optical density and concentration were established and the concentrations of the individual aldehydes determined with these standard curves. The measurements were made using a Hilger Uvispek spectrophotometer.

The radioactivity of the alcoholic solution of the aldehydes was measured with a gas-flow counter (efficiency approx. 17%) and a Phillips scaler No. 4032. For counting, the solutions of the aldehydes were mixed with an equal volume of 2% gelatin in 0·2N NaOH, and 1·0 ml dried evenly on a 1-in. copper disk.

III. Results and Discussion

A sample of the [^{14}C]tyrosine (biosynthesized) used in the earlier work (Dougall and Shimbayashi 1960) was diluted 21 times with non-radioactive tyrosine and was found to have a specific activity of 21,800±1,700 counts per minute (mean and mean deviation of four determinations) per μmole tyrosine. This material on degradation

as described gave *p*-hydroxybenzaldehyde of specific activity 14,700±600 counts per minute (three determinations) per μmole. If the tyrosine was uniformly labelled then the specific activity of the aldehyde should have been 16,900±1,300 counts per minute per μmole. The difference between expected and found specific activities of *p*-hydroxybenzaldehyde should not be regarded as significant because the number of determinations is relatively small.

The data in Table 1 show that the three aldehydes *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde can be obtained from the cell wall residue by oxidation with nitrobenzene. The production of vanillin and syringaldehyde shows that lignin

TABLE 1

YIELDS AND SPECIFIC ACTIVITIES OF THE ALDEHYDES OBTAINED ON OXIDATION OF CELL WALL RESIDUE FROM TOBACCO CALLUS TISSUE WITH NITROBENZENE

Specific activities of the bound tyrosine and of the *p*-hydroxybenzaldehyde which would be obtained from it are included for comparison

Aldehyde	Yield (μg/g)		Specific Activity (counts per minute per μmole)	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
p-Hydroxybenzaldehyde	310	170	20,100±1,400	16,900±1,900
Vanillin	1390	2680	*c.* 400	*c.* 400
Syringaldehyde	230	160	No detectable radioactivity	No detectable radioactivity
Tyrosine (bound)*			8,700± 900	9,200±1,000
p-Hydroxybenzaldehyde from bound tyrosine			5,900± 670	6,700± 670

* From Dougall and Shimbayashi (1960). The values have been corrected for the different counters used.

is present in the cell wall residue. The fact that neither vanillin nor syringaldehyde contained significant amounts of carbon-14 shows that tyrosine is not a precursor of all the phenylpropane residues of the lignin in tobacco callus tissue. Taking the radioactivity of vanillin as a guide to the radioactivity of lignin, then in this case the low recovery of radioactivity by acid hydrolysis of the cell wall fraction cannot be explained by the incorporation of tyrosine into lignin. A similar conclusion was reached by Ibrahim, Lawson, and Towers (1961) who examined the fate of [^{14}C]tyrosine in leaf disks of *Pyrus communis*. These authors obtained an insoluble material which they termed "lignin fraction" by hydrolysis of the 80% ethanol-insoluble material firstly in cold 72% sulphuric acid and then by refluxing in dilute acid. Only 10–12% of the radioactivity of the 80% ethanol-insoluble material was found in this lignin fraction.

The cell wall residue in addition to giving *p*-hydroxybenzaldehyde on nitrobenzene oxidation gave tyrosine on acid hydrolysis. The specific activity of the *p*-hydroxybenzaldehyde isolated was two to three times higher than the value

expected if protein tyrosine was the only source of this aldehyde (see Table 1). It is clear that a second source of *p*-hydroxybenzaldehyde exists in the tobacco callus tissue. The data in Table 1 do not allow the second source of the aldehyde to be identified but they do show that carbon atoms from tyrosine are incorporated more rapidly into this source than into the cell wall protein.

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