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Wheat Lignin Labeling Using [U-^{14}C]Phenylalanine or [*O*-$^{14}CH_3$]Sinapic Acid for Fermentation Studies

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[U-^{14}C]Phenylalanine (Phe*) or [*O*-$^{14}CH_3$]sinapic acid (Sin*) was infused in wheat stems under the node of the apical fraction, with and without leaf, or at midinternode. (^{14}C-Lignin)Pronase-treated cell walls of the upper and lower halves of the internode were prepared and analyzed. With Phe*, the labeling had a greater variability than with Sin* and a different distribution in the organs; cell wall residues were labeled on lignin units with a high specific radioactivity, on phenolic acids, and on residual proteins. Labeling of saponified residues was more lignin-specific. With Sin*, syringyl units were specifically labeled. The infusion at midinternode considerably increased labeling yields, while the elimination of the leaf had very slight effect. Radiochemical and chemical results obtained on internode segments are discussed in terms of lignin labeling validity and lignin biogenesis. These substrates, strongly labeled on complementary structures, are potentially useful tools to study the fate of lignins during ruminal fermentation.

INTRODUCTION

The preparation of cell walls labeled with ^{14}C on lignins (Crawford and Crawford, 1988) is a good means of studying the fate of lignins during cell wall degradation. The transformations undergone by phenolic components during forage digestion by ruminants are minor and probably specific to certain functional groups (Schink, 1988). To assess the extent of these transformations and to determine the mechanisms involved, lignin labeling must be strong, specific to lignins or a lignin function, and representative of the native macromolecule. Also, (^{14}C lignin) cell walls must be well characterized for results of biodegradation to be interpreted unequivocally.

We chose to label lignins in the upper (more lignified) and lower (less lignified) halves of wheat apical internodes, with either [U-^{14}C]phenylalanine (Phe*) or [*O*$^{14}CH_3$]-sinapic acid (Sin*). Phe* was expected to label proteins and the three constitutive units of lignins on the phenylpropanoic carbons, while Sin* was expected to specifically label the syringyl units on one methoxyl.

Lignin labeling has been more thoroughly studied in woods than in grasses (Crawford, 1981). The incorporation of ^{14}C-labeled compounds in wheat has been used in the investigation of lignin biosynthesis since the work of Brown and Neish (1955). Of various precursors, [U-^{14}C]phenylalanine and [2-^{14}C]sinapic acid specifically labeled wheat lignins, but the resulting specific radioactivity of lignin units was low (Brown and Neish, 1956; Higuchi and Brown, 1963a,b). More recently, with [U-^{14}C]phenylalanine as a precursor, (^{14}C lignin) cell walls of wheat apical internodes were prepared to study the aerobic degradation of lignins (Agosin et al., 1985), but labeling of the upper part of the internode was weak and variable (Gaudillere and Monties, 1986).

Labeled sinapic acid is incorporated into lignins more specifically than Phe* but interconversions between lignin units have been observed and vary with the plant species, with the age of the plant, and according to whether

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incorporation is performed in the light or in the dark (Higuchi and Brown, 1963a; Tomimura et al., 1980).

As an expansion of a study of the effect of the dark on wheat lignin labeling (Barthes et al., 1992), this work was undertaken to characterize in detail the labeling of (^{14}C lignin) cell walls of wheat with two complementary precursors and to try to improve labeling yields and labeling distribution along the apical internode. Two approaches were used: the elimination of organs, such as the leaf, liable to dilute the radioactivity (Barnes and Friend, 1975) and the administration of the precursors at a site close to the target stem section.

MATERIALS AND METHODS

Plant Material and Radiochemicals. Spring wheat (Bastion variety) was grown in a field plot to flowering stage.

[U-^{14}C]Phenylalanine (16.65 GBq/mmol) in 0.01 M HCl was purchased from the Commissariat à l'Energie Atomique, Saclay, France. [*O*-$^{14}CH_3$]Sinapic acid (2 GBq/mmol) was synthesized according to the method of Barthes et al. (1991) and dissolved in 0.05 M NaOH before incorporation.

Administration of Precursors. Administration of precursors was performed according to the cut stem procedure (Brown and Neish, 1955)) adapted to wheat plants by Agosin et al. (1985). Briefly, plants were cut under water under the last node (conventional method), and three batches of five apical fractions (stems + leaves + ears + nodes) were placed in small test tubes containing water. The water was sucked off and replaced by the radioactive solution containing 2 MBq of Phe* or Sin*. After 30 min, the uptake of labeled solutions was almost complete and a nutritive solution (0.2 mL) (Coic and Lesaint, 1975) was added as the plant absorbed the liquid. After 6 h, the plant fractions were allowed to metabolize in the dark for 96 h at 25 °C (Barthes et al., 1992) in the nutritive solution. The same procedure was followed with one batch of five apical fractions on which leaves had been removed and with four batches of five upper halves (stems + ears) of the apical fraction after cutting at midinternode. In the last batches, 1 MBq of Phe* or Sin* was administered.

Residue Preparation. The upper and lower halves of the apical internodes (tops and bottoms, respectively) were ground in liquid nitrogen. To remove labeled proteins, the fresh powder was subjected to Pronase digestion (Calbiochem, 2250 units/g of powder in 50 mL of 0.1 M phosphate buffer, pH 7, for 2 h at 35 °C), washed with water, and dried for 48 h at 60 °C (Odier et al., 1981). Cell wall residues (CWR) were prepared by refluxing in 1:2 ethanol–toluene and then in 95° ethanol until the extracts became colorless.

To remove the radioactivity located in esterified phenolic acids, CWR (1 g) were hydrolyzed with 50 mL of 1 M NaOH at 35 °C for 2 h under nitrogen, filtered (sintered glass of porosity 2), and washed with water to yield the saponified residue (SR). Under these conditions, we obtained similar amounts of hydrolyzed coumaric and ferulic acids as in 1 M NaOH at 20 °C for 20 h (Higuchi et al., 1967) but with better physical characteristics of the residue for fermentation studies.

Analysis. Nonradioactive samples were used to assay cellulose, hemicellulose, and lignins (Jarrige, 1961). Hydrolyzed polysaccharides were measured as reducing sugars (Besle et al., 1981). Uncondensed lignin monomers were hydrolyzed in alkaline nitrobenzene according to the method of Venverloo (1971) modified (5 mL of 2 M NaOH, 0.5 mL of nitrobenzene, 160 °C, 3 h, extraction with CH_2Cl_2). Phenolic acids were extracted with ethyl acetate from the alkaline filtrate obtained during SR preparation, according to the procedure of Scalbert et al. (1985).

The alkaline nitrobenzene oxidation products of lignins, *i.e.*, hydroxybenzaldehyde (H), vanillin (V), syringaldehyde (S), and phenolic acids, were analyzed by reversed-phase HPLC according to the method of Scalbert et al. (1985) except that the separation was performed with A and B as eluents (A, $H_2O/CH_3OH/H_3PO_4$ 94/6/0.2; B, CH_3OH/H_3PO_4 100/0.2) with a gradient of 0–30% B from 0 to 20 min at a flow rate of 1.5 mL/min. Fractions of 300 μL were collected to which 5 mL of scintillation fluid (Instagel, Packard) was added before counting. Protein-bound phenylalanine radioactivity was determined according to the method of Attaix et al. (1986) after acid hydrolysis of the CWR (HCl 6 M, 24-h reflux).

Table I. Composition of Wheat Apical Internode

		bottom[a]		top[a]	
		CWR	SR	CWR	SR
residue	% IDM[b]	73.6	35.0	81.8	40.8
		43.7	65.6	43.0	57.5
		33.7	20.5	32.2	25.8
cellulose	% residue	12.4	5.6	12.2	8.2
hemicelluloses					
lignin					
p-coumaric acid (PCA)		0.45	0.0	0.37	0.0
ferulic acid (FA)		0.46	0.0	0.38	0.0
FA/PCA[c]		0.9		0.9	
p-hydroxybenzaldehyde (H)		0.29	0.05	0.25	0.07
vanillin (V)		2.13	0.63	1.95	1.06
syringaldehyde (S)		1.89	0.76	1.49	0.81
S/V[c]		0.74	1.02	0.63	0.64
H + V + S	% lignins	34.8	25.7	30.2	23.6

[a] Top, bottom, upper and lower halves of the apical intermode; CWR, cell wall residue; SR, saponified residue. [b] residue (CWR or SR) expressed in percentage (w/w) of initial dry matter (IDM). [c] Molar ratio. Standard deviations ($n \geq 2$) were lower than 10% except for aldehyde contents (≤15%).

Determination of Radioactivity. The radioactivity of ^{14}C solid samples was estimated with an oxidizer (Packard, Tri-carb 306); that of ^{14}C liquid samples was counted with a liquid scintillation analyzer (Packard, Tri-carb 2000CA).

Statistics. Variance analysis was performed with the GLM procedure (SAS program, 1988) to study the effect of the incorporation method, *i.e.*, leaf elimination and cutting level, on labeling yields measured on plant organs and on cell wall fractions (CWR, SR). Values are given as means ± standard deviations.

RESULTS

Wheat Apical Internode Composition. Cell wall content was 74% initial dry matter (IDM) in the bottoms and 8 percent units higher ($p \leq 0.01$) in the tops of the apical internodes (Table I). In CWR, cellulose, hemicellulose, lignin, and phenolic acid contents were not significantly different in the two halves of the internodes. Saponification removed 52% of the CWR dry matter in the bottoms and 2 percent units less in the tops of the internodes. More lignins were removed ($p \leq 0.01$) in the bottoms than in the tops (79 and 67%, respectively). The same trend was observed with hemicelluloses. Cellulose loss was lower in both sections (on average 31%).

The alkaline nitrobenzene oxidation products were mainly vanillin, syringaldehyde, and a little *p*-hydroxybenzaldehyde. Their contents were notably higher in CWR than in SR. Their total accounted for 35% of CWR lignin content for the bottoms and 5 percent units less for the tops. In SR, they represented a lower proportion of lignins, but the trend from the base upward was the same as in CWR.

Characterization of Lignin Labeling by the Conventional Method. When administration was performed *under the nodes without leaf elimination*, Phe* incorporation yields (radioactivity incorporated percent dose administered) in whole internodes were higher (nonsignificantly) and significantly ($p \leq 0.05$) more variable (71 ± 20%) than Sin* incorporation yields (51 ± 8%) (Table II). We checked that radioactivity remaining in the nutritive solution was negligible after completion of incorporation.

On average, one batch of five apical fractions weighed 4.2 g. The ears, tops, bottoms, leaves, and nodes represented 42.5, 14.3, 14.6, 25.6, and 3% (w/w) of the apical

Table II. Morphological Distribution of Radioactivity into Wheat Apical Internode According to the Site of Incorporation

		[U-^{14}C]phenylalanine		[*O*-$^{14}CH_3$]sinapic acid	
		under node $n = 3$	midinternode $n = 4$	undernode $n = 3$	midinternode $n = 4$
specific radioactivity, Bq/mg	ear	154 ± 62	185 ± 74	166 ± 34	118 ± 16
	top	195 ± 63	645 ± 131	250 ± 18	445 ± 34
	bottom	719 ± 338		556 ± 49	
	leaf	64 ± 23		238 ± 25	
	node	3535 ± 77		718 ± 56	
labeling yield, % total incorporated radioactivity	ear	21 ± 4	43 ± 5	25 ± 4	43 ± 3
	top	8 ± 1	57 ± 5	13 ± 2	57 ± 3
	bottom	29 ± 5		32 ± 3	
	leaf	5 ± 1		23 ± 3	
	node	37 ± 10		8 ± 0.2	
incorporation yield, % dose administered		71 ± 20	73 ± 21	51 ± 8	48 ± 3

Table III. Distribution of Radioactivity in the Cell Wall Fractions of Wheat Apical Internode According to the Site of Incorporation

			[U-^{14}C]phenylalanine		[*O*-$^{14}CH_3$]sinapic acid	
			under node $n = 3$	midinternode $n = 4$	under node $n = 3$	midinternode $n = 4$
specific radioactivity, Bq/mg	CWR	top	93 ± 39	405 ± 74	159 ± 10	288 ± 24
	CWR	bottom	755 ± 351		575 ± 44	
	SR	top	nd[a]	241 ± 33	nd	130 ± 5
	SR	bottom	247 ± 97		265 ± 36	
residual radioactivity, % gross sample radioactivity	CWR	top	38 ± 5	51 ± 4	55 ± 2	53 ± 4
	CWR	bottom	72 ± 1		77 ± 11	
	SR	top	nd	16 ± 1	nd	12 ± 3
	SR	bottom	13 ± 3		17 ± 1	

[a] nd, not determined.

Table IV. Specific Radioactivity (Kilobecquerels per Millimole) of Phenolic Components in Phe*-Labeled Segments[a]

	bottom under node	top midinternode
cell wall residue		
p-coumaric acid (PCA)	1208	117
ferulic acid (FA)	1803	433
FA/PCA	1.5	3.7
saponified residue		
vanillin (V)	1203	386
syringaldehyde (S)	728	462
S/V	0.6	1.2

[a] Standard deviations between triplicates were 15% in the tops and ≤35% in the bottoms.

fractions, respectively. There was little relation between the distribution of labeling in the different organs and their weight with Phe* but slightly more with Sin*. Phe* and Sin* labeling yields (gross sample radioactivity percent total incorporated radioactivity) in the bottoms of the internodes were comparable (*ca.* 30%). In contrast, Sin* was distributed in smaller proportions in the nodes and in larger proportions in the ears than Phe*. Labeling in the leaves was very slight (5%) upon Phe* incorporation but higher (23%) with the incorporation of Sin*.

For both precursors, the proportions of gross sample radioactivity remaining in cell walls (residual radioactivity) (Table III) were generally higher in the bottoms than in the tops. More Sin* was incorporated into cell walls than Phe* (5% more for the bottoms, 17% more for the tops). Cell wall saponification caused a considerable loss of radioactivity in the bottoms (85% for Phe*, 78% for Sin*).

The radioactivity of phenolic components was only measured on the most strongly labeled samples, *i.e.*, in the bottoms (Table IV). That of aldehydes was only determined on SR since in CWR lignin monomers are not analyzed specifically because of the presence of esterified phenolic acids.

In the bottoms of the Phe*-labeled internodes, both phenolic acids and lignin monomers were labeled. Specific radioactivities of *p*-coumaric acid (PCA) and ferulic acid (FA) were high, as were those of vanillin and syringaldehyde. No radioactivity was detected in H units. In addition, we observed that Pronase digestion removed 13% more radioactivity from the cell walls (bottoms) than water extraction and that, even in these conditions, 5% of protein-bound phenylalanine remained in the cell walls.

In the bottoms of the Sin*-labeled internodes, no radioactivity was detected in esterified phenolic acids. In SR, labeling was mainly located in syringaldehyde (81% of collected radioactivity) with a specific radioactivity of 1445 ± 93 kBq/mmol. The remaining radioactivity was concentrated in a peak corresponding to syringic acid (2720 ± 246 kBq/mmol) identified by its retention time and ultraviolet spectrum, though the presence of traces of radioactivity in vanillin cannot be excluded.

Optimization of Lignin Labeling. Since only the site of incorporation had a notable effect on the labeling yields, only those results showing the effect of cutting level are presented (Tables II–IV).

Effect of Leaf Elimination. Administration of precursors under the node with leaf resulted in a relatively small proportion of radioactivity in the leaves, mainly with Phe* as precursor (Table II). *After removal of the leaves* and with Phe* as precursor, this radioactivity was almost totally shifted to the bottoms, since an increase of 4 percent units (nonsignificant) of labeling yields was measured. With Sin*, the greatest increase in labeling yields was observed in the nodes (+10 percent units) with the remaining additional radioactivity being equally spread out in the other organs (+4 percent units, nonsignificant). Moreover, these supplements of radioactivity in gross samples

Table V. Lignin Labeling Characterization into Cell Walls and Saponified Residues of Wheat Apical Internode

	[U-^{14}C]phenylalanine		[O-^{14}CH$_3$]sinapic acid	
	bottom under node	top midinternode	bottom under node	top midinternode
	Percent CWRRA[a]			
p-coumaric acid + ferulic acid	10.6	2.6	0.0	0.0
saponified residue	17.4	30.5	21.6	22.6
alkali-soluble fraction	72.0	66.9	78.3	77.4
	Percent SRRA[b]			
vanillin + syringaldehyde	21.9	18.0	19.2	22.1

[a] CWRRA, cell wall residue radioactivity, alkali-soluble fraction was calculated by difference to 100%. [b] SRRA, saponified residue radioactivity. Standard deviations between triplicates were lower than 15%.

disappeared during chemical fractionation, particularly during saponification, and the specific radioactivities of the cell wall fractions (CWR and SR) were not different from those obtained in samples with leaf.

Effect of Cutting Level. When precursors were administered *at the midinternodes*, incorporation yields were similar to those obtained by incorporation under the nodes (Table II). Labeling yields were considerably increased ($p \leq 0.01$) in the organs, and radioactivity was equally distributed in the tops and the ears for both precursors. The specific radioactivities of the tops of the internodes were then 2 and 3 times higher ($p \leq 0.01$) for Sin* and Phe*, respectively, than in the bottoms. Consequently, specific radioactivities of the cell walls in the tops were considerably increased ($p \leq 0.01$) (Table III). The proportion of residual radioactivity in cell walls of the tops was similar for Phe* and Sin* (*ca.* 52%). With Phe*, this proportion was higher ($p \leq 0.01$) than with incorporation under the nodes (38%).

The alkaline hydrolysis of Phe*-labeled cell walls solubilized less radioactivity in the tops (70%) incorporated at midinternode than in the bottoms (85%) incorporated under the node, while with Sin* the radioactivity solubilized was equivalent in both parts of the internodes (*ca.* 78%).

In the tops incorporated at midinternode, we observed that, like in the bottoms, Phe* labeled phenolic acids and both G and S units (Table IV), while Sin* labeled both syringaldehyde (620 ± 51 kBq/mmol, 78% of recovered radioactivity) and syringic acid (895 ± 74 kBq/mmol). With both precursors, the specific radioactivities were lower ($p \leq 0.01$) in tops than in bottoms, while with Phe*, FA/PCA and S/V specific radioactivity ratios were higher ($p \leq 0.01$) in tops.

To sum up, the results obtained by radiochemical analysis on bottoms after incorporation under the node and on tops after incorporation at midinternode show that the labeling of cell walls was distributed in phenolic acids, saponified residue, and an alkali-soluble fraction, with the two latter fractions containing little labeled protein (case of Phe*) (Table V). The alkali-soluble fraction was the most radioactive and was probably composed of alkali-soluble phenolics. Syringyl-labeled lignins seemed to be slightly more alkali-soluble than uniformly labeled lignins (bottom = 6% and top = 10% more radioactivity solubilized). For both precursors, the percentage of radioactivity solubilized by alkali, excluding phenolic acids (bottom = 72–78%; top = 67–77%), was close to that of alkali-soluble lignins (bottom = 79%; top = 67%) measured by gravimetric methods (Table I). Furthermore, we observed that irrespective of the precursor and the section considered, monomer radioactivity determined by nitrobenzene oxidation represented on average 20% of saponified residue radioactivity. This percentage was not far from that determined by gravimetric analysis (bottom = 26%; top = 24%) (Table I).

DISCUSSION

In recent studies, Lam et al. (1990a,b, 1992) and Iiyama et al. (1991) observed that the phenolic chemical composition of four sections of wheat apical internode reflected the maturation of cell walls from the base upward. Our results were consistent with their findings on several points: (1) the difference in cell wall content between bottoms and tops; (2) the alkali solubility of lignins, already observed by Lapierre et al. (1989a), which was higher in bottoms than in tops; (3) the slight decrease in the total yield of alkaline nitrobenzene oxidation products from bottoms to tops, which itself reflects an increase in condensed lignins. In contrast, the few differences in lignin content and FA/PCA and S/V ratios along the internode were probably due to the large size of the stem sections studied. In addition, the relatively low content in hydroxybenzaldehyde in cell wall and saponified residues, compared to that in vanillin and syringaldehyde, was also observed by Lapierre et al. (1989b) in more mature wheat plants in which aldehyde contents were higher.

The radioactivity losses measured during Phe* and Sin* incorporation were also found by Higuchi and Brown (1963a) and may be due at least partly to metabolization of the precursors by the plant (El Basyouni et al., 1964a). The lack of migration of phenylalanine evidenced by its distribution in the organs has already been observed in wheat leaf by Barnes and Friend (1975). Sin* migration along the stem was faster than that of Phe*. The higher residual radioactivity in cell walls of bottoms and tops obtained upon Sin* incorporation under the node can be explained by its better migration and because Sin* is a closer precursor of lignins. The reason residual radioactivity in cell walls of tops was higher when Phe* was administered midinternode rather than under the node may result from the low migration of Phe* from the base upward, involving a delay in its incorporation into lignins when administration was performed under the node. However, given that incorporation lasted 96 h, the effect of migration must have been minor since the incorporation of (β-^{14}C)cinnamate into lignins of 35-day-old wheat stems was achieved in 24 h (El Basyouni et al., 1964b). The dose rate of Phe*, 4 times higher in tops incorporated at midstem, is a more likely explanation of the higher conversion of Phe* into lignins, and as Watkin and Neish (1960) have shown, the percentage of Phe* conversion depends on the optimal dose rate.

Phe* and Sin* incorporation into lignins resulted in several reactions which were enzyme-catalyzed. Unlike Higuchi (1969), we did not measure the activities of the enzymes involved, but our results provided information on lignin biogenesis in young (bottoms) and old (tops) cell walls. If we consider the incorporation yields and the

labeling distribution, the dose of each precursor was equivalent in the tops incorporated at the midinternodes (Phe* = 419 kBq and Sin* = 278 kBq) and in the bottoms incorporated under the nodes (Phe* = 410 kBq and Sin* = 328 kBq). However, with both precursors, residual radioactivity in cell walls was still lower in the tops (*ca.* 50%) than in the bottoms (>70%). In parallel, the specific radioactivities of uncondensed lignin monomers and esterified phenolic acids were much lower in the tops than in the bottoms of the internodes. These results on the metabolization of precursors suggest a higher lignification activity in the bottoms than in the tops. In contrast, with Phe* as precursor, S/V and FA/PCA specific radioactivity ratios were higher in the tops than in the bottoms. Gaudillere and Monties (1986) observed the same trend in the S/V ratio using wheat apical internodes at the same stage. Hence, there seems to be a difference in the lignification pattern between tops and bottoms, with a possible tendency toward the production of greater methoxylated end products in tops.

Leaf elimination did not prevent the migration of the precursors along the stem and their incorporation into cell walls. However, its slightly positive effect on labeling yields of bottoms and tops was not clear-cut because of the marked variability of cell wall labeling, even with Sin*, which was the best incorporated in the leaves and had the least variability. We showed that administration of the precursors at a site close to the target stem section resulted in a strong lignin labeling both in bottoms and in tops, the first aim of this work. Cell wall labeling yields and the specific radioactivities of lignin monomers were much higher in the sections studied than when precursors were administered in whole young plants (Higuchi and Brown, 1963a,b).

Reliable lignin labeling, which is essential for fermentation studies, depends first on the specificity of the labeling and second on the similarity of the labeled and native lignins. The radioactivity of Klason lignins was not directly measured in this work since a fraction of the radioactivity of Phe*-labeled cell walls of wheat is solubilized in acid medium (Agosin et al., 1985). This radioactivity was more likely composed of acid-soluble lignins and proteins rather than carbohydrates (Crawford and Crawford, 1983). Unlike Agosin et al. (1985), we found some radioactivity in proteins of Phe*-labeled CWR after Pronase hydrolysis. With Sin* as precursor, labeling of carbohydrates and proteins was unlikely. This precursor was expected to label specifically one of the three constitutive units of lignins. Thus, little or no conversion of syringyl units into guaiacyl units was observed. This is consistent with the results of Brown and Neish (1963a,b), who observed decreasing interconversions with increasing age of the wheat. According to Barthes et al. (1992), this labeling specificity was not due to the effect of incorporation in the dark. The highly labeled syringic acid was adequately separated from vanillin in contrast with previously reported data (Barthes et al., 1992). This acid may have resulted from the alkaline nitrobenzene oxidation of syringaldehyde or from sinapic acid β-oxidation by the plant (El Basyouni et al., 1964a). In the latter case, syringic acid must have been strongly linked to lignins because it resisted the mild alkaline treatment applied for saponified residue preparation.

Given the different methods used, especially for the determination of lignin content and radioactivity, these results obtained by radiochemical and chemical analysis were in sufficient agreement to suggest that (i) besides known contamination, the labeling occurs on its lignin target and (ii) newly synthesized labeled lignins have a structure close to that of native lignins. This second finding needs confirmation; in particular, the slightly higher degree of condensations of the newly synthesized (^{14}C)lignins than the native ones may support the observation of Iyama et al. (1991) on young and mature wheat lignins. However, according to the results of Scobbie et al. (1993), a maize internode lignifies for less than 1 week. The length of time (4 days) used to prepare labeled lignins may therefore be comparable to the length of time taken by lignification of the apical internode. In addition, the work of Terashima and Fukushima (1989), who visualized lignification processes in hardwood xylem cell walls by histoautoradiography, suggests that labeled lignins are deposited simultaneously in cells of different maturity and should therefore have a structural heterogeneity close to that of native lignins.

Among the (^{14}C lignin) cell wall substrates, those prepared with Phe* may be regarded as complementary to those prepared with Sin*; both have specific radioactivities sufficient for fermentation studies. Extensive characterization of both substrates is an important first step in the interpretation of fermentation results.

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