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METABOLIC DIFFERENCES BETWEEN GYMNOSPERMS AND ANGIOSPERMS IN THE FORMATION OF SYRINGYL LIGNIN

YOSHIKI NAKAMURA, HIDEHUMI FUSHIKI and TAKAYOSHI HIGUCHI

Division of Lignin Chemistry, Wood Research Institute, Kyoto University, Uji, Kyoto, Japan

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Abstract—Sliced xylem tissue from shoots of both poplar and cherry reduces ferulic and sinapic acids to the corresponding aldehydes and alcohols, while tissue from gymnosperms such as Japanese red pine and ginkgo can reduce only ferulic acid. In young, less differentiated, xylem tissue and callus tissue of angiosperms the ability to reduce sinapic acid is markedly lower than that of the fully differentiated xylem.

Both gymnosperm and angiosperm tissues reduced coniferyl and sinapyl aldehydes to the corresponding alcohols and, further, the peroxidases from both classes gave similar dehydrogenation polymers from a mixture of coniferyl and sinapyl alcohols. In agreement with these findings, sinapyl aldehyde and sinapyl alcohol, when fed to living plants and tissue cultures of gymnosperms, were shown to be readily converted to syringyl lignin which was not originally present.

INTRODUCTION

IT IS well known that gymnosperm lignins consist almost entirely of guaiacyl units, whereas angiosperm lignins contain, in addition, syringyl moieties. The following biosynthetic pathway to syringyl lignin has been established, mainly by tracer experiments;[1–3] ferulic acid(FA) → 5-hydroxyferulic acid(5-HFA) → sinapic acid(SA) → sinapyl alcohol → syringyl lignin. It is considered, therefore, that one or more enzymes mediating these reactions are not present in gymnosperms, and in fact, 5-HFA is hardly methylated to SA by gymnosperm *O*-methyltransferase (OMT).[4]

The present paper describes differences in the ability to metabolize syringyl compounds between gymnosperms and angiosperms, and the artificial formation of syringyl lignin when sinapyl aldehyde and sinapyl alcohol are fed to gymnosperms. The results are discussed in relation to controling factors in the formation of guaiacyl and syringyl lignins.

RESULTS

Reduction of FA and SA with sliced tissues

Slices of fresh shoots of poplar (*Populus nigra*) and cherry (*Prunus yedoensis*) were incubated in neutral buffered solutions of FA and SA (each 10 mM), and the amounts of coniferyl and sinapyl aldehydes, and of coniferyl and sinapyl alcohols formed after 5 hr were

[1] BROWN, S. A. and NEISH, A. C. (1959) *J. Am. Chem. Soc.* **81**, 2419.
[2] HIGUCHI, T. and BROWN, S. A. (1963) *Can. J. Biochem. Physiol.* **41**, 613.
[3] SHIMADA, M., FUSHIKI, H. and HIGUCHI, T. (1972) *Phytochemistry* **11**, 2247.
[4] SHIMADA, M., KURODA, H. and HIGUCHI, T. (1973) *Phytochemistry* **12**, 2873.

examined. Table 1 (Expt. 1) shows a remarkable increase in the amount of coniferyl aldehyde and coniferyl alcohol in these tissues. However, the amount of sinapyl alcohol in poplar, which was fed with SA, was smaller than that of the control but larger than that in the tissue fed with FA. Separate experiments indicate that when the tissues were fed with SA at concentrations of 0–10^{-2} M, there was an increased amount of sinapyl alcohol, but little change in sinapyl aldehyde (Fig. 1). The results also show that coniferyl alcohol increases to some extent, which suggests the occurrence of a demethoxylation step of SA to FA as described previously[1,2,5] and subsequent reduction of the FA formed to coniferyl alcohol.

TABLE 1. EFFECT OF FERULIC AND SINAPIC ACIDS ON THE AMOUNTS OF THE CORRESPONDING ALDEHYDES AND ALCOHOLS IN VARIOUS PLANT TISSUES

		Compound obtained (μg/g fr. wt)			
Plant material	Compound fed (mM)	Coniferyl aldehyde	Coniferyl alcohol	Sinapyl aldehyde	Sinapyl alcohol
Expt. 1. Differentiated angiosperm					
Poplar	Control	0	4	Tr	24
(fresh shoot)	FA (10)	3	123	Tr	9
	SA (10)	0	17	Tr	17
Cherry	Control	0	5	—*	—
(fresh shoot)	FA (10)	8	314	—	—
	SA (10)	0	6		—
Expt. 2. Less differentiated angiosperm					
Mulberry	Control	0	Tr	0	0
(tissue culture)	FA (5)	0	10	—	—
	SA (5)	—		0†	0†
Poplar	Control	0	0·3	0	0
(etiolated shoot)	FA (5)	0	14	—	—
	SA (5)	—	—	0†	0†
Expt. 3. Differentiated gymnosperm					
Japanese red pine	Control	0	5	0	0
(fresh shoot)	FA (10)	0·6	34	0	0
	SA (10)	0	9	0	0
Ginkgo biloba	Control	Tr	L.	0	0
(fresh shoot)	FA (10)	Tr	V.L.	0	0
	SA (10)	Tr	L.	0	0
Expt. 4. Less differentiated gymnosperm					
Cryptomeria japonica	Control	0	0·3	0	0
(tissue culture)	FA (10)	0	15	0	0
	SA (10)	0	0·4	0†	0†
Japanese black pine	Control	0	1·0	0	0
(seedling)	FA (5)	0	20	—	—
	SA (5)	—	—	0†	0†

Tr = trace; V.L. = very large amount; L. = large amount.
* Not examined.
† Incubation was run in duplicate.

Table 1 (Expt. 2) further shows that mulberry callus tissue (*Morus bombycis*) and etiolated poplar shoots, neither of which contained any significant amount of syringyl lignin and gave a negative Mäule colour test, did not give any sinapyl aldehyde and sinapyl alcohol when fed with SA, but did show increases in coniferyl alcohol from FA.

[5] STEINER, A. M. (1970) *Z. Pflanzenphysiol.* **63**, 370.

Similar experiments were carried out with gymnosperms (Table 1, Expts. 3 and 4). When FA was supplied to tissue of Japanese red pine shoots (*Pinus densiflora*), the amount of coniferyl alcohol increased several fold as compared with that of control. On the other hand, SA solution externally supplied did not cause any production of sinapyl aldehyde and sinapyl alcohol, but resulted in a slight increase in coniferyl alcohol in agreement with the earlier isotopic evidence.[6] The less differentiated *Cryptomeria japonica* callus tissue and Japanese black pine seedlings (*P. thunbergii*) behaved similarly.

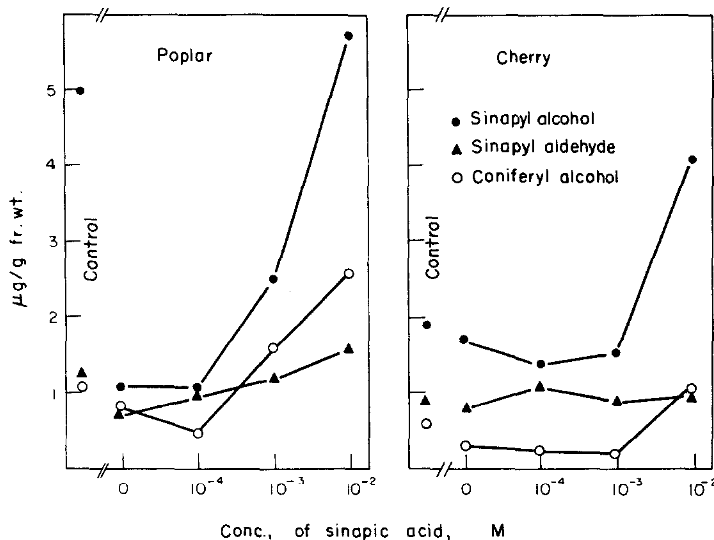

FIG. 1. EFFECT OF VARIOUS CONCENTRATIONS OF SINAPIC ACID ON THE AMOUNTS OF SINAPYL AND CONIFERYL ALCOHOLS AND SINAPYL ALDEHYDE IN SLICED TISSUES OF POPLAR AND CHERRY SHOOTS.

Reduction of coniferyl and sinapyl aldehydes with sliced tissues and cell free extracts

The above results and early tracer experiments[7] suggest that coniferyl and sinapyl aldehydes are intermediates between the corresponding acids (FA and SA) and alcohols, and this was recently supported by the enzyme experiments of Gross *et al.*[8] To establish the differences in the ability of gymnosperms and angiosperms to reduce coniferyl and sinapyl aldehydes to the corresponding alcohols, sliced and callus tissues of both classes were supplied with either coniferyl or sinapyl aldehydes. It can be seen that gymnosperms as well as angiosperms reduce both coniferyl and sinapyl aldehydes to the corresponding alcohols, the direct precursors of guaiacyl and syringyl lignins in angiosperms (Table 2).

The reduction of both aldehydes with crude enzyme solutions prepared from callus tissues of *C. japonica* and etiolated poplar shoots was found to be specific for NADPH in contrast to NADH specific yeast ADH[9,10] (Table 3). It is considered, therefore, that such

[6] HIGUCHI, T. (1962) *Can. J. Biochem. Physiol.* **40**, 31.
[7] HIGUCHI, T. and BROWN, S. A. (1963) *Can. J. Biochem. Physiol.* **41**, 621.
[8] GROSS, G. G., STÖCKIGT, J., MANSELL, R. L. and ZENK, M. H. (1973) *FEBS Letters* **31**, 283.
[9] SUND, H. and THEORELL, H. (1963) *The Enzymes* (BOYER, P. D., LARDY, H. and MYRBÄCK, K., eds.), Vol. 7, p. 25, Academic Press, New York.
[10] BARMAN, T. E. (1969) *Enzyme Handbook*, Vol. 1, p. 23, Springer, Berlin.

reducing ability may be due to the unspecific nature of the aromatic alcohol dehydrogenases (aromatic ADH) specific for NADPH which were reported recently by Davies *et al.*[11]

TABLE 2. EFFECT OF CONIFERYL AND SINAPYL ALDEHYDES ON THE AMOUNTS OF THE CORRESPONDING ALCOHOLS IN TISSUES OF GYMNOSPERMS AND ANGIOSPERMS

Plant material	Compound fed (5 mM)	Compound obtained (μg/g fr. wt) Coniferyl alcohol	Sinapyl alcohol
Cryptomeria japonica (tissue culture)	Control	0·3	0
	Coniferyl aldehyde	32*	†
	Sinapyl aldehyde	—	44*
Japanese black pine (seedling)	Control	1·0	0
	Coniferyl aldehyde	292	—
	Sinapyl aldehyde	—	156
Mulberry (tissue culture)	Control	0·1	0
	Coniferyl aldehyde	42*	
	Sinapyl aldehyde	—	77*
Poplar (etiolated shoot)	Control	0·3	0
	Coniferyl aldehyde	142	—
	Sinapyl aldehyde	—	23‡

* A mixture solution of coniferyl and sinapyl aldehydes was fed.
† Not examined.
‡ Browning occurred in sliced tissues.

Preparation of dehydrogenation polymer (DHP) with peroxidase

No previous investigation has been carried out to determine whether the polymerization of the *p*-hydroxycinnamyl alcohols, the final step of lignification, is affected by different enzymes in gymnosperms and angiosperms. This question was investigated by observing

TABLE 3. COFACTOR SPECIFICITY OF ALCOHOL DEHYDROGENASES IN THE REDUCTION OF CONIFERYL AND SINAPYL ALDEHYDES TO THE CORRESPONDING ALCOHOLS

	Source of enzyme (product formed, μmoles)								
	Cryptomeria japonica (tissue culture)			Poplar (etiolated shoot)			Yeast		
Substrate*	A	B	A/B	A	B	A/B	A	B	A/B
Coniferyl aldehyde	1·10	0·57	1·9	0·80	0·17	4·7	0·44	1·25	0·35
Sinapyl aldehyde	0·90	0·05	18·0	0·90	0·17	5·3	~0	1·16	~0

* 1·5 μmol of each used.
A = alcohols formed with NADPH; B = alcohols formed with NADH.

the degree of polymerization of a mixture of coniferyl and sinapyl alcohols using peroxidases extracted from black pine seedlings and mulberry callus tissue respectively. The polymer obtained using pine seedling peroxidase gave the following monomers on acidolysis: vanillin; guaiacylacetone; vanilloyl methyl ketone (VMK); 2-hydroxy-1-(3-methoxy-4-hydroxyphenyl)-1-propanone; ω-hydroxyguaiacylacetone; syringylacetone; 1-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-2-propanone; 2-hydroxy-1-(3,5-dimethoxy-4-hydroxy-

[11] DAVIES, D. D., UGOCHUKWU, E. N., PATIL, K. D. and TOWERS, G. H. N. (1973) *Phytochemistry* **12**, 531.

phenyl)-1-propanone; ω-hydroxysyringylacetone. This indicates that gymnosperm peroxidase can polymerize both coniferyl and sinapyl alcohols simultaneously, producing a polymer containing both guaiacyl and syringyl groups. Angiosperm peroxidase from mulberry callus tissues gave a very similar result. Although the enzyme preparations used were relatively crude, it can be concluded that the peroxidases from both gymnosperms and angiosperms have a common activity in the polymerization of the *p*-hydroxycinnamyl alcohols.

TABLE 4. EFFECT OF VARIOUS SYRINGYL COMPOUNDS ON THE AMOUNTS OF SYRINGOYL METHYL KETONE DERIVED FROM SYRINGYL LIGNIN FORMED IN GYMNOSPERMS

Ginkgo biloba (fresh shoot)		*Cryptomeria japonica* (tissue culture)	
Compound fed (mg)	SMK (μg/g dry wt of plant residue)	Compound fed (mg)	SMK (total μg*)
Control	0	Control	0
SA (80)	0	SA (120)	0
SA (100) + FA (30)	0	SA (170) + FA (50)	0
Sinapyl aldehyde (100)	170	Sinapyl aldehyde (120)	123
Sinapyl alcohol (100)	173	Sinapyl alcohol (120)	216
Sinapyl alcohol (150) + coniferyl alcohol (50)	192	Sinapyl alcohol (170) + coniferyl alcohol (50)	180

* As a large portion of syringyl compounds fed was absorbed into agar media, the amount of SMK obtained from *Cryptomeria japonica* was much smaller than that of *Ginkgo biloba* as shown.

Artificial formation of syringyl lignin in gymnosperms

Previous work[4] and the results obtained in the present investigation showed that cell free extracts of gymnosperm tissues could effectively catalyze both the reduction of sinapyl aldehyde to sinapyl alcohol and the dehydropolymerization of the alcohol, although the activities of gymnosperm enzymes participating in the methylation of 5-HFA and in the reduction of SA were negligible. It is conceivable, therefore, that if sinapyl aldehyde and sinapyl alcohol were externally supplied to gymnosperms, they might well be incorporated into a syringyl lignin by the mediation of aromatic ADH and peroxidase. Accordingly, sinapyl aldehyde, sinapyl alcohol and SA were fed to fresh shoots of *Ginkgo biloba* and callus tissues of *C. japonica*, and then the acidolysis monomers with syringyl groups from the cell wall residues were analyzed (Table 4). In all specimens guaiacylacetone and VMK produced from the endogenous guaiacyl lignin were found in the largest amounts. In addition, the samples fed with sinapyl aldehyde or sinapyl alcohol yielded syringylacetone and syringoyl methyl ketone (SMK), which were not found from the control samples. On the other hand, SA did not give any syringyl compounds. These results conclusively indicate that even in gymnosperms, syringyl lignin can be formed from sinapyl aldehyde and sinapyl alcohol but not from SA.

DISCUSSION

It is evident that the biosynthetic pathway to syringyl lignin, including the reduction step of SA, is operative in differentiated angiosperm tissue. Zenk *et al.*[8,12] have recently demonstrated that FA is reduced to coniferyl aldehyde via ferulyl–CoA thioester with the extracts of cambial tissues of *Salix alba* and *Forsythia*. It seems probable that, SA is similarly reduced to sinapyl aldehyde through the corresponding CoA ester in angiosperms.

[12] MANSELL, R. L., STÖCKIGT, J. and ZENK, M. H. (1972) *Z. Pflanzenphysiol.* **68**, 286.

Since this reduction is not found in gymnosperms, it appears that the enzymes required for the formation of sinapyl–CoA from SA and/or its reduction to sinapyl aldehyde are not present.

Except the reduction step of SA, both gymnosperms and angiosperms gave very similar results in the metabolism of syringyl compounds. The demethoxylation of SA to FA and subsequent reduction of the FA to coniferyl alcohol were found in both classes of plants. Higuchi[6] obtained radioactive VMK but no SMK from tissue cultures of white pine (*Pinus strobus*) fed with SA-[2-^{14}C]. It seems possible that all gymnosperms may have ability to convert substances such as SA into FA, which can be utilized to form lignin.

Sinapyl aldehyde was reduced to sinapyl alcohol, which was further incorporated into gymnosperm lignins because the aromatic ADH and peroxidase of gymnosperms have a broad spectra of substrate specificity. Davies *et al.*[11] isolated an aromatic ADH from potato tubers, although they did not test its activity with coniferyl and sinapyl aldehydes. Gross *et al.*[8] found that the enzyme from *Forsythia* catalyzes the reversible reduction of coniferyl aldehyde and is specific for NADPH. This information and the results in Table 3 suggest the important role of the aromatic ADH (E.C. 1.1.1.2) in lignin biosynthesis.

Syringylacetone and SMK have been established to be derived from the syringylglycerol-β-aryl ether structure of syringyl lignin.[13] The occurrence of syringylacetone and SMK in the acidolysis products of the plants fed with sinapyl aldehyde, thus indicates that the aldehyde was reduced to sinapyl alcohol which was then converted to a syringyl lignin by the mediation of peroxidase. The amounts of SMK found were not influenced by the simultaneous supply of guaiacyl compounds such as FA and coniferyl alcohol, although it was reported that dehydropolymerization of sinapyl alcohol alone gave syringaresinol rather than a polymer containing β-aryl ether groups.[14] The amount of sinapyl alcohol which contributed to the β-aryl ether moieties in ginkgo lignin was estimated to be 3–4% of the compound fed.[13]

The lack of syringyl units in gymnosperm lignins can be explained in terms of the negligible activity of the reduction of SA as well as the low activity of gymnosperm OMT's methylating 5-HFA to SA.[15] However, the decreased amounts of syringyl lignin in young xylem tissue and callus tissue of angiosperms could not be explained in terms of the substrate specificity of their OMT's.[15] Because bamboo and angiosperm callus OMT's mediated the formation of FA and SA equally, regardless of the degree of differentiation of the tissue from which the enzymes were extracted. The reduction of SA was not found in less differentiated angiosperms as well as in gymnosperms, while both FA and SA were reduced by fully differentiated angiosperms. Consequently, it seems that the increase in syringyl lignin in angiosperm tissue followed by the progress of maturation is due to the production of SA reducing enzymes during xylem differentiation.

EXPERIMENTAL

Plant materials. Fresh shoots of poplar (*Populus nigra*), cherry (*Prunus yedoensis*), Japanese red pine (*Pinus densiflora*) and ginkgo (*Ginkgo biloba*) grown in the campus of this Institute were used. *Ginkgo* was also used for studies of the formation of syringyl lignin. Callus tissues of *Cryptomeria japonica* and mulberry (*Morus bombycis*) were cultured at 27 in the light for 70 90 days using the media of Heller[16] and of Murashige and Skoog.[17]

[13] HIGUCHI, T., TANAHASHI, M. and NAKATSUBO, F. (1973) *Wood Res.* **54**, 9.
[14] FREUDENBERG, K. and BRITTNER, F. (1950) *Chem. Ber.* **83**, 600.
[15] SHIMADA, M., FUSHIKI, H. and HIGUCHI, T. (1973) *Mokuzai Gakkaishi* **19**, 13.
[16] HELLER, R. (1953) Thèse Fac. Sci. Paris.
[17] MURASHIGE, T. and SKOOG, F. (1962) *Physiol. Plantarum* **15**, 473.

respectively. Seedlings of Japanese black pine (*P. thunbergii*) germinated as previously reported[18] were used for the reduction of FA and SA and for the extraction of peroxidase. Branches of 5-yr-old poplar were cut into about 50 cm in length and the severed ends were placed in H_2O at 27° in dim light for 3–4 weeks. Partly etiolated yellowish shoots, grown up 10–20 cm, were sliced and used for the reduction of the acids and aldehydes.

Chemicals. Both FA and SA were synthesized according to the method of Brown.[19] Coniferyl and sinapyl aldehydes were synthesized as reported previously.[20] Coniferyl and sinapyl alcohols were synthesized by the method of Freudenberg.[21]

Feeding experiments. (a) Reduction of FA and SA with slices tissues. Sliced tissues (15 g) of fresh shoots of poplar, cherry, red pine and *Ginkgo* were immersed for 5 hr in 10 ml of either H_2O or aq. solns of FA and SA neutralized with $NaHCO_3$ (pH 7·0–7·5). Sliced tissues of the etiolated poplar shoots, excised hypocotyls of black pine seedlings, and disintegrated callus tissues of *Cryptomeria* and mulberry were examined in the same way. In each experiment, a batch of tissues was immediately subjected to EtOH extraction designated as control. (b) Reduction of the aldehydes with sliced tissues. Each 5 g of callus tissues of *Cryptomeria* and mulberry, black pine seedlings and sliced tissues of the etiolated poplar shoots were incubated at 30° for 4 hr in solns of coniferyl and sinapyl aldehydes (15 μmol/3 ml for callus tissues and 30 μmol/6 ml for other plants). (c) Formation of syringyl lignin in gymnosperms. Solns of sinapyl aldehyde, sinapyl alcohol and SA (1%) were fed to fresh cut shoots of *Ginkgo*, and the shoots left for 3 days. Similarly, a mixture of sinapyl and coniferyl alcohols or SA and FA was fed to *Ginkgo* shoots. Aq. EtOH solns of each compound and each mixture (1%) were added aseptically to the callus tissue of *Cryptomeria* which was incubated at 27°.

Analyses of reduction products. Plant tissues were homogenized in 95% hot EtOH. The EtOH extracts were taken to dryness, dissolved in hot H_2O (10 ml) and then extracted with Et_2O (15 ml × 4). The Et_2O extracts were submitted to preparative TLC on silica gel PF_{254} (Merk) with Et_2O-*n*-hexane (2:1, v/v) or Et_2O–$CHCl_3$ (1:1, v/v). The aldehydes and alcohols, which were detected under UV light, were extracted with 5% MeOH in $CHCl_3$ or acetone and the extracts were converted to their TMS-derivatives and subjected to GC–MS (Shimazu-LKB 9000). The compounds gave the identical retention times and MS with those from authentic compounds. Simultaneously, the amounts of the products formed were determined by GLC with SE-52 (3%) column at 210–218°.

Analyses of acidolysis products. Fresh shoots of *Ginkgo*, fed with syringyl compounds, were cut into small pieces and homogenized in acetone, and the cell wall residues were dried *in vacuo*. The residues (3·0–5·5 g) were subjected to acidolysis at reflux temp. for 24 hr[13] and the acidolysis products were chromatographed on a silica gel column (1·7 × 3 cm). The column was eluted with $CHCl_3$ (25 ml) and $CHCl_3$–EtOAc (9:1, v/v) (15 ml), successively. The eluate was evaporated to dryness *in vacuo*, dissolved in 0·5 ml $CHCl_3$, and submitted to GC–MS. The retention times and MS of guaiacylacetone, VMK, syringylacetone and SMK were identical with those of the authentic specimens, respectively. Quantitative analysis of SMK was performed by GLC (SE-52, 3% column, 205–210°). Acidolysis of callus tissues of *Cryptomeria* was carried out in a small scale in a sealed glass tube and the acidolysis products were analyzed in the same way.

Extraction and assay of aromatic ADH. Each 12 g of callus tissues of *Cryptomeria* and sliced tissues of the etiolated poplar shoots were homogenized at about 4° in a mortar with an eq. wt of 0·1 M phosphate buffer (pH 7·5), containing cysteine, NaN_3, mercaptoethanol (5 mM each), polyclar AT (2 g) and bovine albumin (200 mg), respectively. The homogenate was filtered through a cheese cloth and the filtrate was centrifuged at 0° for 10 min (12700 *g*). The supernatant soln was passed through a Sephadex G25 column (1·7 × 15 cm), equilibrated with 0·01 M phosphate buffer (pH 7·5) containing 3 mM mercaptoethanol. The eluate was collected and used for the assay of aromatic ADH activity. Yeast ADH (Boehringer Mannheim) was also examined under the same condition for comparison. The reaction mixture contained the following components in a final vol. of 2·5 ml; 1·5 μmol of coniferyl or sinapyl aldehyde, 5 μmol of NADH or NADPH, 2 ml of the plant enzyme solns or 1·2 mg protein of yeast ADH and 25 μmol of phosphate buffer (pH 7·5). The reaction mixture was incubated at 30° for 2 hr. The reaction product was subsequently extracted with Et_2O (10 ml × 4), the Et_2O was evaporated, and the residue was submitted to preparative TLC. Coniferyl and sinapyl alcohols separated on the plates were extracted with 5% MeOH in acetone and determined by measurement of the absorbances at 265 and 275 nm, respectively. The relative activity of aromatic ADH was expressed by the amount of alcohols produced.

Extraction of peroxidase and preparation of DHP of coniferyl and sinapyl alcohols. Each 40 g fresh weight of mulberry callus and pine seedlings were cooled in liquid N_2 and ground in a mortar. The ground tissue was extracted with 40 ml of 0·05 M phosphate buffer (pH 6·0), filtered through a cheese cloth and centrifuged for 15 min at 12700 *g*. To the supernatant soln was added solid $(NH_4)_2SO_4$ to 0·75 saturation, and the ppt. was collected by centrifugation. The ppt. was dissolved in a small amount of the same buffer in a cellophane tube and dialysed overnight at 4°. The crude peroxidase thus obtained was used for the preparation of DHP of coniferyl and sinapyl alcohols. A mixture soln of coniferyl alcohol (112 mg) and sinapyl alcohol (130 mg) in 100 ml of 0·1 M phosphate buffer (pH 6·0) and 42 ml of 0·1% H_2O_2 soln in respective separatory funnels were added

[18] Shimada, M., Fushiki, H. and Higuchi, T. (1972) *Phytochemistry* **11**, 2657.
[19] Brown, S. A. and Neish, A. C. (1955) *Can. J. Biochem. Physiol.* **33**, 948.
[20] Nakamura, Y., Nakatsubo, F. and Higuchi, T. (1974) *Wood Res.* **56**, 1.
[21] Freudenberg, K. and Hübner, H. H. (1952) *Chem. Ber.* **85**, 1181.

dropwise to 20 ml of the enzyme soln in a beaker for 1 hr with stirring. Then, the reaction was allowed to continue for 23 hr further at room temp., a second 20 ml of enzyme soln being added to the reaction mixture after 10 hr. The turbid soln was centrifuging for 15 min at 9000 *g*, the ppt. was dissolved in a small amount of acetone, and the acetone soln added dropwise into 10 vol. of H_2O with stirring. The precipitated polymer was collected by centrifuging for 15 min at 9000 *g*. Acidolysis of polymer was carried out as described above except that the reaction time was 4 hr, and the acidolysis monomers were identified as TMS-derivatives comparing their MS with those of authentic specimens by GC–MS.

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