Phytochemistry, Vol. 31, No. 11, pp. 3801–3806, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00
Pergamon Press Ltd

STRUCTURE–FUNGICIDAL PROPERTIES OF SOME 3- AND 4-HYDROXYLATED STILBENES AND BIBENZYL ANALOGUES

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(*Received in revised form* 7 *April* 1992)

Key Word Index—*Gloeophyllum trabeum*; *Poria placenta*; *Coriolus versicolor*; stilbenes; fungicidal activity; structure–activity relationship; pinosylvin; pinosylvin monomethyl ether.

Abstract—The fungicidal activity of some substituted hydroxylated stilbenes and bibenzyls was measured against three wood-destroying fungi using the agar plate technique. Four 3-hydroxystilbene derivatives and 3-hydroxybibenzyl were active against the white-rot fungus *Coriolus versicolor*. A tentative structure–activity relationship was proposed for stilbene *C. versicolor* activity. The previously proposed hypothesis that white-rot extracellular enzymes oxidatively degrade stilbenes which makes white-rot fungi immune to stilbenes may need to be reexamined. No structure–activity relationship was apparent for stilbenes tested against the two brown-rot fungi *Gloeophyllum trabeum* and *Poria placenta*. Of the two natural hydroxylated stilbenes studied, pinosylvin was slightly more active against *G. trabeum* and *C. versicolor* than pinosylvin monomethyl ether. Surprisingly, neither of these two natural stilbenes showed much activity against the copper-tolerant fungus *P. placenta*.

INTRODUCTION

Hydroxylated stilbenes are formed by woody plants both as constitutive biocidal agents and as stress-formed metabolites (phytoalexins). Consequently, stilbenes and related compounds have been extensively studied to determine their role in disease and decay resistance of plants and wood products [1–4]. Stilbenes are produced in a broad spectrum of plants via both the acetate and shikimic acid pathways [1, 2]. The ring synthesized by the acetate pathway often has a 3′,5′-dihydroxylated structure. One or both of these phenolic groups may be further methylated. The shikimic acid-derived ring may be unsubstituted or can be substituted with various hydroxyl and/or methoxyl group(s) which are usually located in the 4; 3 and 4; or 3, 4 and 5 positions. While the *trans* (*E*) isomer is usually the only isomer isolated, a few investigators have reported isolating small amounts of *cis* (*Z*) isomers [5–7]. These *cis* isomers may be formed during isolation [2] by a photolytic *trans/cis* isomerization [8] and consequently may be artifacts.

Stilbenes isolated from decay-resistant wood have been shown to be fungitoxic when tested in agar with no wood present. However, these same compounds imparted little or no fungicidal activity when impregnated into nondurable wood [2, 3]. These results have been interpreted to suggest that stilbenes act in combination with the other extractives present in decay-resistant wood. We recently suggested another possibility which may act in concert with synergism. Specifically, we proposed that when stilbenes are first formed in plants they may have only moderate activity [9, 10]. However, the stilbene biosynthesis pathway can be altered to form biorelated compounds, or the newly formed stilbene can be directly modified *in vivo*, to give derivatives with greater and/or broader activities [1, 9, 10].

In prior studies, we examined the fungicidal properties of (*E*)-3′- and/or 4′-substituted 4-hydroxystilbenes (Fig. 1), related bibenzyls, and oxidatively polymerized dimers by the agar plate and soil block techniques [9, 10]. In this study, we report on the fungicidal properties of 3- and/or 4- and/or 5-substituted stilbenes and a few related bibenzyls against brown- and white-rot fungi using the agar plate method. The prime ring is unsubstituted in this study, in contrast to our earlier work [9, 10] where the prime ring contained various substituents (Fig. 1). When one stilbene was synthesized both the *cis* and *trans* isomers were isolated and consequently the geometric isomer influence on bioactivity was examined. In addition to the synthesized stilbenes three natural stilbenes, pinosylvin and its mono- and dimethyl ethers, were also studied. These synthesized compounds were studied because of their similarity to pinosylvin and also to examine the substituent effect on the opposite ring from our earlier QSAR studies.

RESULTS AND DISCUSSION

The compounds examined in this study are listed in Table 1 and the IC_{50} fungicidal values in Table 2.

White-rot fungus

Four 3-hydroxylated stilbenes (**3**, **4**, **12** and **13**) and 3-hydroxybibenzyl (**5**) showed fungicidal activity

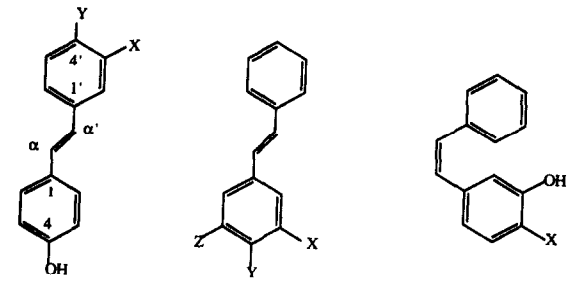

Fig. 1. Structure and numbering of stilbenes in this study and prior work.

against *C. versicolor* (Table 2). No stilbene had white-rot activity in our previous studies [9, 10]. A tentative structure–activity relationship for *C. versicolor* activity may involve a 3-hydroxystilbene with either an unsubstituted or substituted 5-position.

While some researchers have suggested that stilbenes in general have no white-rot activity [4, 11], other reports indicate that stilbenes are active against one or more white-rot fungi [12–15]. One reason often mentioned for white-rot fungi being unaffected by stilbenes is that the extracellular oxidative enzymes produced by white-rot fungi [16] may convert stilbenes into non-fungicidal compounds [2, 12, 14, 17–19]. The relative ease by which 4-hydroxystilbenes can be oxidatively polymerized with peroxidase/H_2O_2 [9, 20, 21] and our prior studies [9, 10]

Table 1. Compounds tested for fungicidal properties

No.	Abbreviation	Name
1	4-OH	(*E*)-4-Stilbenol
2	4-OH RED*	4-Hydroxybibenzyl
3	3-OH	(*E*)-3-Stilbenol
4	*cis*-3-OH	(*Z*)-3-Stilbenol
5	3-OH RED*	3-Hydroxybibenzyl
6	4-OH, 3-OMe	(*E*)-3-Methoxy-4-stilbenol
7	4-OH, 3-OMe RED*	4-Hydroxy-3-methoxybibenzyl
8	4-OH, 3,5-diOMe	(*E*)-3,5-Dimethoxy-4-stilbenol
9	4-OH, 3-Cl	(*E*)-3-Chloro-4-stilbenol
10	3,4-diOH	(*E*)-3,4-Stilbenediol
11	*cis*-3-OH, 4-OMe	(*Z*)-4-Methoxy-3-stilbenol
12	3,5-diOH	(*E*)-3,5-Stilbenediol (pinosylvin)
13	3-OH, 5-OMe	(*E*)-5-Methoxy-3-stilbenol (pinosylvin monomethyl ether)
14	3,5-diOMe	(*E*)-3,5-Dimethoxystilbene (pinosylvin dimethyl ether)

*RED indicates a stilbene which has been reduced to a bibenzyl.

Table 2. Concentration in parts per million at which the fungus growth is inhibited by 50% relative to the fungus growth in untreated agar

		IC_{50} (ppm)		
		Brown-rot fungi		White-rot fungus
No.	Compound	*G. trabeum*	*P. placenta*	*C. versicolor*
1	4-OH*	31*	12*	>350*
2	4-OH RED*	38*	20*	224*
3	3-OH	35†	54†	49
4	*cis*-3-OH	65	25†	75†
5	3-OH RED	44	49	78†
6	4-OH, 3-OMe	40†	40†	>350†
7	4-OH, 3-OMe RED	>90†‡	13	>350
8	4-OH, 3,5-diOMe	60	49	>350
9	4-OH, 3-Cl	43†	40†	>350†
10	3,4-diOH	34	33	>350†‡
11	*cis*-3-OH, 4-OMe	64	8†	>350
12	3,5-diOH	29	>90‡	140
13	3-OH, 5-OMe	42	>90‡	163
14	3,5-diOMe	>90	>90‡	>350

*Data from prior paper [10].
†Average of two or more activity values.
‡Some activity was observed at the highest concentration examined.

where we found that no 4-hydroxystilbene was bioactive against *C. versicolor* support this oxidative hypothesis. Thus, 4-hydroxystilbenes would be anticipated to be inactive against white-rot fungi. In addition, neither *cis* nor *trans* geometric isomers would be protected against oxidation. Consequently, no significant fungicidal difference would be expected between a *trans* (**3**) or *cis* (**4**) isomer, as was observed (Table 2). Finally, white rot extracellular enzymes are known to be powerful oxidants. Thus, it is not unexpected that these enzymes can oxidize stilbenes.

This hypothesis, that the extracellular enzymes of white-rot fungi can oxidatively degrade stilbenes into nontoxic compounds, unfortunately does not account for all the data in this or prior studies. First, many prior studies have reported that stilbenes which are oxidatively polymerized *in vivo* are bioactive [22–26]. Consequently, oxidative polymerization does not necessarily inactivate a stilbene, although other modes of oxidative degradation besides polymerization are possible. Also, one stilbene without a 4-hydroxyl (**11**) had no *C. versicolor* activity, and thus the absence of a 4-hydroxyl does not always indicate white-rot activity. Second, reduction of the stilbene to the corresponding bibenzyl should inhibit oxidative degradation either through polymerization or cleavage of the double bond. In prior work [10] we found that four of the 12 bibenzyls tested had activity against a white rot fungus and one of the two bibenzyls synthesized for this study had white-rot activity (Table 2). Consequently, our data suggest that reduction of the double bond does not necessarily give the corresponding bibenzyl white-rot activity. Finally, the above hypothesis suggests that stilbenes are fungitoxic against white-rot fungi until degraded by extracellular enzymes. However, enzymatic degradation requires that the white-rot fungus first produce extracellular enzymes which then must diffuse to and degrade the stilbene, all of which requires time. Since the agar plate test is relatively rapid (measurements taken at 4 and 5 days for *C. versicolor*), some fungal inhibition would be anticipated by stilbenes which are initially fungitoxic prior to being oxidatively degraded. The fact that no inhibition was observed for most stilbenes in the short-term agar plate test may indicate that such stilbenes are simply not fungitoxic to white-rot fungi at the concentrations examined.

The fact that certain stilbenes are inhibitory to white rot fungi does not imply that these same stilbenes cannot eventually be degraded by white-rot extracellular enzymes. Thus, fungitoxic stilbenes which are present in sublethal concentrations may eventually be detoxified/degraded by white-rot fungus [14].

The data in Table 2 and prior studies [12–15] show that a few stilbenes with a specific structure have white-rot fungicidal activity. A hypothesis to explain why only some stilbenes have white-rot fungicidal properties, consistent with all available data, cannot be suggested. Consequently, the previously proposed hypothesis that white-rot extracellular enzymes can oxidatively degrade stilbenes into inactive compounds and thus stilbenes are not fungitoxic to white-rot fungi [2, 12, 14, 17–19] may need to be reexamined.

Brown-rot fungi

All hydroxylated stilbenes examined in this study (Table 2) had moderate brown-rot activity. Interestingly, the substitution pattern of the stilbenes appears to have little influence on brown-rot activity (Table 2). Only small differences in fungicidal properties were observed with *G. trabeum*. While the *P. placenta* data varied more, some of this may be due to the variable results often observed with *P. placenta*. Interestingly, our prior studies [9, 10] found that the 3'- and/or 4'-substitution pattern (Fig. 1) determined the presence or absence of and also the level of brown-rot activity. This study suggests that substitution of the 3- or 5-position(s) of 4-hydroxystilbene (Fig. 1) has little effect on brown-rot activity. Substitution in the 4- or 5-position of 3-hydroxystilbene has some influence on activity, but its nature is unclear.

The three bibenzyls tested in this study also had moderate brown-rot activity, but the limited number studied makes interpretation of the data difficult. *cis/trans* Isomers appear to have somewhat similar fungicidal properties for *G. trabeum* and *C. versicolor*, although this is based on only one pair of isomers (**3** vs **4**). The isomer effect is less clear for *P. placenta*. Other studies have also reported that *cis/trans* stilbenes have similar fungicidal properties [2, 6, 27].

The two natural hydroxylated stilbenes **12** (pinosylvin) and **13** (pinosylvin monomethyl ether) gave somewhat different results. Against *G. trabeum*, and also the white-rot *C. versicolor*, **12** was slightly more active than **13**. Prior studies [3, 12–14] have reported that **12** is usually more fungitoxic than **13**. Against the copper-tolerant fungus *P. placenta*, however, only limited activity at the highest level tested of 60 ppm was observed for **12** and **13**. In our prior studies [9, 10] we found that all stilbenes which were active against *G. trabeum* were even more active against *P. placenta*. However, in this study all three (*E*)-3-hydroxystilbenes which had no 4-substituent (**3**, **12**, and **13**) were less active against *P. placenta* than *G. trabeum*. It is possible that whatever makes *P. placenta* copper tolerant also gives this fungus some tolerance against stilbenes with a specific structure.

The fully methylated natural stilbene **14** [5] had little or no activity against the three fungi examined. This result would be anticipated for a stilbene with no free hydroxyl [2, 6, 9, 10, 27], and **14** has been previously reported to be non-fungicidal [3]. However, brown-rot fungi are able to demethylate the methoxyl groups of lignin [28, 29]. It may be possible that brown-rot fungi are also capable of demethylating **14** to form the fungitoxic stilbenes **12** and **13**. This possible bioactivation would take some time and may not be observed in the short-term test of the agar-plate method.

EXPERIMENTAL

Stilbenes were synthesized using previously published methods [9, 30], and selected stilbenes reduced with 30 psi H_2 over Pd/C to form the corresponding bibenzyl. Mp: uncorr. Elemental analyses were performed by Galbraith. Pinosylvin monomethyl ether (**13**) was donated by Hercules Chemical Company and purified by silica gel chromatography. Pinosylvin and pinosylvin dimethyl ether were synthesized using previously published methods [31].

Biological testing by the agar plate method was performed as described earlier [9] using the brown-rot fungi *Poria placenta* (Fr.) Cook, and *Gloeophyllum trabeum* (Pres, ex Fires) Murr and the white-rot fungus *Coriolus versicolor* (L.) (Quél). Stilbene concentrations used were 3, 6, 15, 30 and 60 ppm for the two

Table 3. ^{13}C and ^{1}H NMR spectral data for compounds **3**–**7** and **9**–**13** (in acetone-d_6)

C	3	4	5	6	7	9	10	11	12	13
1	139.8	139.5	144.2	130.5	133.9	131.6	130.2	130.0	140.4	140.4
2	113.9	115.2	116.2	110.2	112.8	127.2	113.8	111.2	106.0	107.0
	7.09	6.77 (*t*)	6.6–6.8 (*m*)	7.25	6.77	7.55	7.12 (*d*)	6.77 (*s*)	6.59 (*d*)	6.69 (*m*)
3	158.6	158.2	158.2	148.6	147.9	121.5	146.2	146.0	159.6	159.6
4	115.6	116.2	113.6	147.6	145.5	153.4	146.2	146.7	103.1	101.9
	6.77 (*ddd*)	6.71	6.6–6.8 (*m*)						6.31 (*t*)	6.36 (*t*)
5	130.5	130.1	130.0	115.9	115.5	117.8	116.2	115.3	159.6	162.2
	7.18 (*t*)	7.06 (*t*)	7.08 (*t*)	6.83 (*d*)	6.74 (*d*)	7.03 (*d*)	6.82 (*d*)	6.78 (*d*)		
6	118.9	120.8	120.4	121.2	121.5	127.2	120.0	120.5	106.0	104.3
	7.09 (*dd*)	6.71	6.6–6.8 (*m*)	7.05	6.65 (*dd*)	7.41 (*dd*)	6.94 (*dd*)	6.70 (*dd*)	6.59 (*d*)	6.69 (*m*)
α	129.5	130.8	38.5	129.7	38.2	128.1	129.6	130.2	129.8	129.5
	7.18 (*s*)	6.57 (*s*)	2.85 (*m*)	7.15 (*d*)	2.82 (*m*)	7.14 (*s*)	7.08 (*d*)	6.49 (*s*)	7.10 (*s*)	7.17 (*d*)
α′	129.3	131.1	38.4	126.7	38.9	127.9	126.5	128.5	129.2	129.6
	7.18 (*s*)	6.57 (*s*)	2.85 (*m*)	7.10 (*d*)	2.86 (*m*)	7.14 (*s*)	7.00 (*d*)	6.49 (*s*)	7.10 (*s*)	7.15 (*d*)
1′	138.3	138.1	142.7	138.8	142.7	138.4	138.8	137.6	138.4	138.3
2′	127.3	129.0	129.2	127.0	129.2	128.6	127.0	128.6	127.3	127.4
	7.58 (*dd*)	7.15–7.25	7.15–7.27	7.54 (*d*)	7.1–7.3 (*m*)	7.59 (*dd*)	7.53 (*dd*)	7.15–7.30	7.5 (*dt*)	7.58 (*dt*)
3′	129.5	129.6	129.0	129.5	128.9	129.5	129.4	128.1	129.5	129.5
	7.36 (*t*)	7.15–7.25	7.15–7.27	7.34 (*t*)	7.1–7.3 (*m*)	7.35 (*t*)	7.34 (*td*)	7.15–7.30	7.36 (*tt*)	7.36 (*tt*)
4′	128.4	127.9	126.5	127.8	126.5	128.2	127.8	126.9	128.3	128.4
	7.26 (*tt*)	7.15–7.25	7.15–7.27	7.22	7.1–7.3 (*m*)	7.24 (*tt*)	7.21 (*tt*)	7.15–7.30	7.25 (*tt*)	7.26 (*tt*)
OMe	—	—	—	56.3	56.1	—	—	55.3	—	55.5
				3.90 (*s*)	3.75 (*s*)			3.78 (*s*)		3.78 (*s*)
(OH)	(8.4)	—	(8.22)	—	(8.48)	(8.9)	(7.97, 8.10)	—	(8.3)	(8.4)

brown rot fungi and 25, 75, 150 and 300 ppm for the white rot fungus. Five replicates were run on each fungus/compound/concentration combination. The radial mycelial growth was measured on the fourth and fifth day after inoculation for *C. versicolor*, sixth and seventh day for *G. trabeum*, and ninth and tenth day for *P. placenta*.

All NMR spectra were obtained at 25° ± 1° observing ^1H and ^{13}C at 300.67 and 75.61 MHz, respectively. NMR samples contained *ca* 40 mg of each compound in 0.5 ml of Me_2CO-d_6, 1% in TMS. Me_2CO-d_6 was chosen as the solvent because of the large solvent shifts [32] it induces in aromatic protons. All chemical shifts were referenced to internal TMS. Typical values of the coupling constants found in these stilbenes were $J_{ortho} = 8.0 \pm 0.5$ Hz, $J_{meta} = 2.2 \pm 0.2$ Hz and $J_{trans} = 16 \pm 0.5$ Hz. The chemical shifts of the *cis* alkenyl hydrogens were identical so no coupling was observed for them.

Complete assignments of the ^1H and ^{13}C NMR spectra were possible from a variety of 1 and 2D techniques, including H, H-COSY [33], CSCM [34], CSCMLR [35–37], NOE difference [38] and INAPT [39] studies. The methodology used to make the assignments in Table 3 is given in our earlier NMR study of some (*E*)-3′- and 4′-substituted stilben-4-ols [40].

(*E*)-4-Hydroxystilbene (**1**); mp 187–188° [9]. 4-Hydroxybibenzyl (**2**); mp 99–100° [9]. (*E*)-3-Hydroxystilbene (**3**); mp 119–122° (lit. [30] 121–122°). (*Z*)-3-Hydroxystilbene (**4**); bp 132–140°/2 m torr. (lit. [30] 130–140°/14 m torr). 3-Hydroxybibenzyl (**5**); mp 75–76°. Found: C, 84.59%; H, 7.09%. $C_{14}H_{14}O$ requires: C, 84.81%; H, 7.12%. IR ν_{max}^{KBr} cm^{-1}: 1590, 1480, 1440 (Ar); 864, 787, 698 (Ar-H). EIMS (probe) 70 eV, *m/z* (rel. int.): 198 $[M]^+$ (14), 107 $[M-ArCH_2]^+$ (57); 91 $[M-HOArCH_2]^+$ (100). (*E*)-4-Hydroxy-3-methoxystilbene (**6**); mp 129–130° (lit. [41] 132.5–133.5°). 4-Hydroxy-3-methoxybibenzyl (**7**); bp 143°/15 m torr. Found C, 79.11%; H, 7.32%. $C_{15}H_{16}O_2$ requires: C, 78.92%; H, 7.06%. IR ν_{max}^{KBr} cm^{-1}: 1604, 1516, 1496 (Ar); 1271, 1237 (Ar-O); 1030 (–OMe); 814, 793 (Ar-H). EIMS (probe) 70 eV, *m/z* (rel. int.): 228 $[M]^+$ (18), 137 $[M-ArCH_2]^+$ (100). (*E*)-3,5-Dimethoxy-4-hydroxystilbene (**8**); mp 121–122°. Found: C, 75.32%; H, 6.38%. $C_{16}H_{16}O_3$ requires: C, 74.98%; H, 6.29%. IR ν_{max}^{KBr} cm^{-1}: 1580, 1482 (Ar); 1240, 1200 (Ar-O); 1024 (–OMe), 970 (*trans* –C=C–). EIMS (probe) 70 eV, *m/z* (rel. int.): 256 $[M]^+$ (75), 149 $[M-107]^+$ (25); 107 $[M-149]^+$ (100). (*E*)-3-Chloro-4-hydroxystilbene (**9**); mp 122–124°. Found: C, 73.96%; H, 5.18%; Cl, 14.28%. $C_{14}H_{11}ClO$ requires: C, 72.89%; H, 4.81%; Cl, 15.37%. IR ν_{max}^{KBr} cm^{-1}: 1540, 1490 (Ar); 1295 (Ar-O), 980 (*trans* –CH=CH–). EIMS (probe) 70 eV *m/z* (rel. int.): 230 $[M]^+$ (100), 194 $[M-Cl]^+$ (48); 165 $[M-65]^+$ (71). (*E*)-3,4-Dihydroxystilbene (**10**); mp 163–166° (lit. [21] 168–169°). (*Z*)-3-Hydroxy-4-methoxystilbene (**11**); bp 147–153°/3 m torr. Found: C, 79.43%; H, 6.20%; OMe, 13.76%. $C_{14}H_{11}O(OCH_3)$ requires: C, 79.62; H, 6.24%; OMe, 13.71%. IR ν_{max}^{KBr} cm^{-1}: 1586, 1520, 1449 (Ar), 1245 (Ar-O), 1019 (–OMe), 704 (*cis* –HC=CH–). EIMS (GC) 70 eV, *m/z* (rel. int.): 226 $[M]^+$ (100); 165 $[M-61]^+$ (95). Attempts to purify the small amount of the *trans* (*E*) isomer were unsuccessful. (*E*)-3,5-Dihydroxystilbene (**12**); mp 153–154° (lit. [31] 155–155.5°). (*E*)-3-Hydroxy-5-methoxystilbene (**13**); mp 119–120° (lit. [31] 118.5–119.5°). (*E*)-3,5-Dimethoxystilbene (**14**); mp 55–56° (lit. [31] 55–56°).

Acknowledgements—This work was supported by USDA-Wood Utilization Research Grant 88-34158-3330, USDA-Competitive Grant 88-33521-4090, the State of Mississippi, and National Science Foundation-EPSCoR Grant RII-8902064. The authors thank Dr James Minn of Hercules Chemical Company (Hattiesburg, MS) for providing compound **13**.

REFERENCES

1. Kindl, H. (1985) in *Biosynthesis and Biodegradation of Wood Components*, Chap. 14 (Higuchi, T., ed.). Academic Press, New York.
2. Hart, J. H. (1981) *Ann. Rev. Phytopathol.* **19**, 437.
3. Hart, J. H. and Hillis, W. E. (1974) *Phytopathology* **64**, 939.
4. Hart, J. H. and Shrimpton, D. M. (1979) *Phytopathology* **69**, 1139.
5. Rowe, J. W., Bower, C. L. and Wagner, E. R. (1969) *Phytochemistry* **8**, 235.
6. Keen, N. T. and Ingham, J. H. (1976) *Phytochemistry* **15**, 1794.
7. Fernandez, M. I., Pedro, J. R. and Seoane, E. (1983) *Phytochemistry* **22**, 2819.
8. March, J. (1977) *Advanced Organic Chemistry* 2nd Edn. McGraw–Hill, New York.
9. Schultz, T. P., Hubbard, T. F., Jr, Jin, L., Fisher, T. H. and Nicholas, D. D. (1990) *Phytochemistry* **29**, 1501.
10. Schultz, T. P., Cheng, Q., Boldin, W., Hubbard, T. F., Jr, Jin, L., Fisher, T. H. and Nicholas, D. D. (1991) *Phytochemistry* **30**, 2939.
11. Lyr, H. (1961) *Enzymologia* **23**, 231.
12. Rennerfelt, E. (1945) *Svensk Bot. Tidskrift* **39**, 311.
13. Rennerfelt, E. and Nacht, G. (1955) *Svensk Bot. Tidskrift* **49**, 419.
14. Loman, A. A. (1970) *Can. J. Botany* **48**, 1303.
15. Woodward, S. and Pearce, R. P. (1988) *Physiol. Mol. Plant Pathol.* **33**, 127.
16. Eriksson, K.-E. L., Blanchette, R. A. and Ander, P. (1990) *Microbial and Enzymatic Degradation of Wood and Wood Components*. Springer, Berlin.
17. Lyr (1962) *Nature* **195**, 289.
18. Scheffer, T. C. and Cowling, E. B. (1966) *Ann. Rev. Phytopathol.* **4**, 147.
19. Rosch, V. R. (1965) *Holz Organismen* **1**, 173.
20. Langcake, P. and Pryce, R. J. (1977) *J. Chem. Soc., Chem. Commun.* 208.
21. Donnelly, D. M. X., Murphy, F. G., Polonski, J. and Prange, T. (1987) *J. Chem. Soc., Perkin Trans. I* 2719.
22. Pryce, R. J. and Langcake, P. (1977) *Phytochemistry* **16**, 1452.
23. Langcake, P. and Pryce, R. J. (1977) *Experientia* **33**, 151.
24. Powell, R. G., Bajaj, R. and McLaughlin, J. L. (1987) *J. Nat. Prod.* **50**, 293.
25. Bokel, M., Diyasena, M. N. C., Gunatilaka, A. A. L., Kraus, W. and Sotheeswaran, S. (1988) *Phytochemistry* **27**, 377.
26. Kawabata, J., Mishima, M., Kurihara, H. and Mizutani, J. (1991) *Phytochemistry* **30**, 645.
27. Ward, E. W. B., Unwin, C. H. and Stoessl, A. (1975) *Can. J. Botany* **53**, 964.
28. Kirk, T. K. and Adler, E. (1970) *Acta Chem. Scand.* **24**, 3379.
29. Jin, L., Schultz, T. P. and Nicholas, D. D. (1990) *Holzforschung* **44**, 133.
30. Dershem, S. M., Fisher, T. H., Johnson, S. and Schultz, T. P. (1988) *Holzforschung* **42**, 163.
31. Bachelor, F. W., Loman, A. A. and Snowdon, L. R. (1970) *Can. J. Chem.* **48**, 1554.
32. Laszlo, P. (1967) *Prog. Nucl. Magn. Reson. Spectr.* **3**, 231.
33. Bax, A., Freeman, R. and Morris, G. A. (1981) *J. Magn. Reson.* **42**, 164.
34. Bodenhausen, G. and Freeman, R. (1978) *J. Am. Chem. Soc.* **100**, 320.
35. Freeman, R. and Morris, G. A. (1978) *J. Chem. Soc., Chem. Commun.* 684.
36. Zektzer, A. S., John, B. K. and Martin, G. E. (1987) *Magn. Res. Chem.* **25**, 752.

37. Johnson, L. F. (1989) *Relaxation Times* **6**, 2.
38. Neuhaus, D. and Williamson, M. (1989) *The Nuclear Overhauser Effect in Structural and Conformational Analysis.* VCH, New York.
39. Bax, A. (1984) *J. Magn. Reson.* **57**, 314.
40. Fisher, T. H. and Schultz, T. P. (1991) *Magn. Res. Chem.* **29**, 966.
41. Sinsheimer, J. E. and Smith, R. V. (1967) *J. Pharm. Sci.* **56**, 1280.