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CIS-MONOLIGNOLS IN *FAGUS GRANDIFOLIA* AND THEIR POSSIBLE INVOLVEMENT IN LIGNIFICATION

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Key Word Index—*Fagus grandifolia*; Fagaceae; American beech; biosynthesis; *cis/trans*-monolignols; lignin; *p*-hydroxycinnamyl alcohols; dehydrogenative polymerized lignin.

Abstract—Lignification in all plant species is assumed to occur exclusively via the dehydrogenative polymerization of the *trans* (*E*) monolignols, *p*-coumaryl, coniferyl and sinapyl alcohols. This assumption may have to be revised somewhat due to the presence of both *E* (*trans*) and *Z* (*cis*) isomers of *p*-hydroxy substituted cinnamic acids in grasses, and *cis*-coniferyl and *cis*-sinapyl alcohols in beech bark (*Fagus grandifolia*). This suggests that lignification of these tissues may use either *cis*- or *trans*-monolignols. By means of H_2O_2/peroxidase induction, we have prepared synthetic dehydrogenative polymerized (DHP) lignin from both *cis*- and *trans*-coniferyl alcohols. Under the tests employed, the degradation products from both DHPs were identical suggesting that either *cis*- or *trans*-monolignols are suitable substrates for this enzyme and, therefore, lignification. An alternative hypothesis is that the *cis*-monolignols accumulate in beech bark because they are not suitable substrates. Therefore, it would follow that the enzymes involved in lignification *in vivo* are highly specific.

INTRODUCTION

Cell wall-bound hydroxycinnamic acids in grasses (Poaceae) grown under normal dark/light cycles, exist as mixtures of *trans* (*E*) (**1–3**) and *cis* (*Z*) (**4–6**) isomers [1–4]. This is in contrast to dark-grown plants, where only the *trans*-isomers are evident [1, 4]. These findings have been explained by results from *in vitro* studies, i.e. when hydroxycinnamic acids **1–3** were exposed to UV irradiation [5–7], sunlight or laboratory lighting [8], a photochemically induced *cis/trans*-isomerization occurred to give mixtures of acids **1–6**. At equilibrium, these solutions contain *ca* 20–30% of the *cis*-isomers.

Cell wall-bound acids in graminaceous plants are predominantly feruloyl and *p*-coumaroyl esters attached to the hemicellulose matrix. The linkage of ferulic acid to hemicellulose has recently been reported in wheat bran [3], sugar cane bagasse [9] and maize [10]. Bound esters of this type have been postulated as precursors of grass lignin [11–13].

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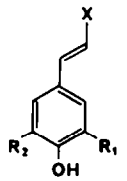
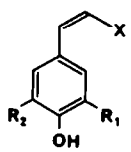




1. $R_1, R_2 = H$, $X = CO_2H$
2. $R_1 = OCH_3$, $R_2 = H$, $X = CO_2H$
3. $R_1, R_2 = OCH_3$, $X = CO_2H$
7. $R_1, R_2 = H$, $X = CH_2OH$
8. $R_1 = OCH_3$, $R_2 = H$, $X = CH_2OH$
9. $R_1, R_2 = OCH_3$, $X = CH_2OH$

4. $R_1, R_2 = H$, $X = CO_2H$
5. $R_1 = OCH_3$, $R_2 = H$, $X = CO_2H$
6. $R_1, R_2 = OCH_3$, $X = CO_2H$
10. $R_1, R_2 = H$, $X = CH_2OH$
11. $R_1 = OCH_3$, $R_2 = H$, $X = CH_2OH$
12. $R_1, R_2 = OCH_3$, $X = CH_2OH$
13. $R_1 = OCH_3$ $R_2 = H$, $X = CH_2O$ Glu-

According to current dogma, lignins arise *exclusively* via oxidative coupling of the *trans*-monolignols **7–9**. It was therefore important to note that both the *cis* (*Z*) form of coniferyl alcohol (**11**), and its glucoside, faguside (**13**), occur in beech bark (*Fagus silvatica*) [14]. No mention was made of the presence, or the relative amounts, of the corresponding *trans* (*E*) isomer (**8**).

To account for the formation of **11**, either a photochemically- or enzymatically-induced isomerization must be occurring at some stage in the conversion of phenylalanine to coniferyl alcohol. The presence of this *Z*-isomer suggests that beech bark lignin may arise, at least in part, from *cis*-(*Z*)-coniferyl alcohol (**11**). On the other hand, monolignol **11** may simply be a metabolite which is not involved in lignin biosynthesis.

In this study, monolignols were extracted and isolated from beech wood and bark, as well as from other plant species representative of grasses, angiosperms and gymnosperms. In addition, synthetic dehydrogenative polymerized (DHP) lignin was prepared, using either *cis*- or *trans*-coniferyl alcohol, and chemically degraded to determine whether the stereochemistry of the monolignols produced had been affected.

RESULTS AND DISCUSSION

Most lignin investigations are carried out with bark-free wood which has been depleted of both organic and hot-water soluble materials by solvent extraction. Subsequent treatment of various pre-extracted woods with THF–water (1:1) at 180° for 50 min results in the release of small quantities of monolignols, presumably in the *E* (*trans*) configuration, i.e. compounds **7–9** [15–17]. It is generally accepted that these alcohols, released during this hydrolytic treatment, are chemically bonded to the lignin macromolecule as 'end groups' [15–17]; the most common bonding patterns suggested being phenolic linkages to either α [15–17] or β [18] functionalities of the lignin polymer (Fig. 1).

In our study, each plant was freshly harvested and bark separated from wood at the cambium layer. In the case of bamboo (*Bambusa tulda gramineae*), greening stem tissue was separated from the vascular bundles. Each tissue sample was then air-dried, ground to a fine powder, and a portion sequentially extracted with hexane, methanol and water. The resulting extracted material was then treated with THF–water; all extracts were then analysed for their monolignol contents. For purposes of comparison, unextracted plant material was also treated with THF–water and the resulting soluble material analysed as before.

Extraction of beech wood with hexane and methanol afforded only minute quantities of the *trans*-(*E*)-monolignols **8** and **9**; no *cis*-isomers were observed. In a similar manner, when either pre-extracted or unextracted beech wood was treated with THF–water, only the *trans*-(*E*)-monolignols were released. These findings are in agreement with our established understanding of lignin formation, i.e. it occurs via a dehydrogenative, free-radical, polymerization of the *trans*-monolignols **7–9**.

On the other hand, the hexane and methanol extracts of beech bark only contained *cis*-coniferyl (**11**) and *cis*-sinapyl (**12**) alcohols; no *trans*-isomers were detected. A minor constituent ($\leq 3\%$) having the same HPLC retention as trans-coniferyl alcohol (**8**) was detected, but was conclusively shown not to be this material (by NMR evidence). Subsequent treatment of this pre-extracted bark with THF–water (1:1) only afforded small quantities of the corresponding *trans*-(*E*)-isomers **8** and **9**. When unextracted beech bark was heated with THF–water as before, mixtures of all four monolignols **8**, **9**, **11** and **12** were obtained.

Thus, since only *cis*-monolignols are found in the hexane/methanol extracts, it can be postulated that lignification in beech bark occurs via the dehydrogenative polymerization of *cis*-alcohols **10–12**. The resulting lignin should be more or less identical to that produced from the corresponding *trans*-isomers in wood. This is because the resonance stabilized radicals from both *E*/*Z* isomers are separated only by a small energy barrier, when delocalization is extended to the olefinic functionality (Scheme 1). Consequently, THF–water treatment of a lignin built up from either *cis*- or *trans*-monolignols should give similar, if not identical, monolignol degradation products. To verify this hypothesis, we synthesized artificial dehydrogenative polymerized (DHP) lignin from both [*cis* (**11**) and *trans* (**8**)] coniferyl alcohols.

cis-Alcohol **11** was synthesized from vanillin (Scheme 2) as follows. Treatment of the ethoxyethylidene [19, 20] derivative **14** of vanillin with carbon tetrabromide, triphenylphosphine and zinc dust [21] in methylene chloride gave, after reprotection, the dibromo-olefin **15**. Reaction of olefin **15** with 2.5 equivalents of *n*-BuLi in THF, followed by quenching with methyl chloroformate, gave acetylenic ester **16**. The required *cis*-olefinic ester **17** was then cleanly prepared by catalytic hydrogenation using freshly prepared Lindlar catalyst [22]. Reduction of

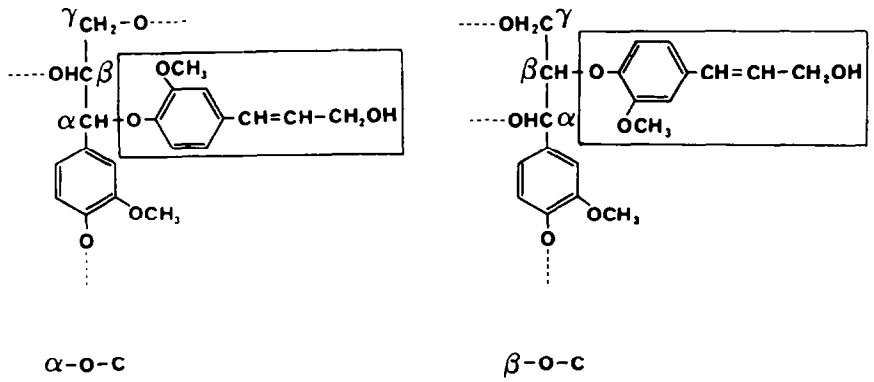
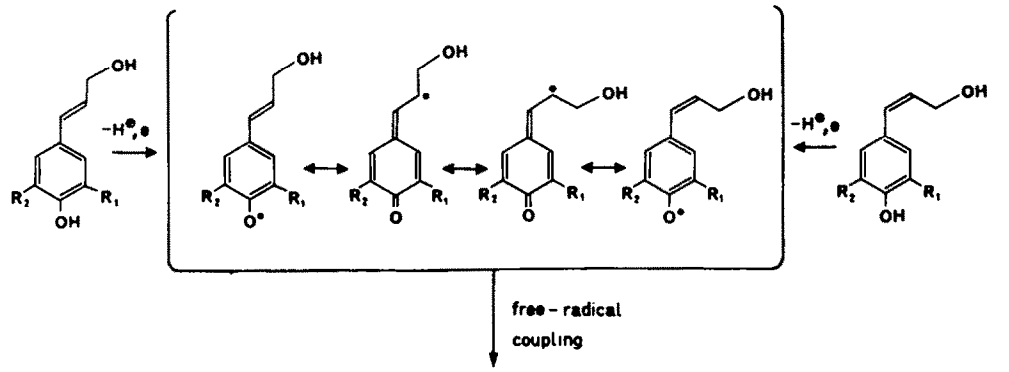


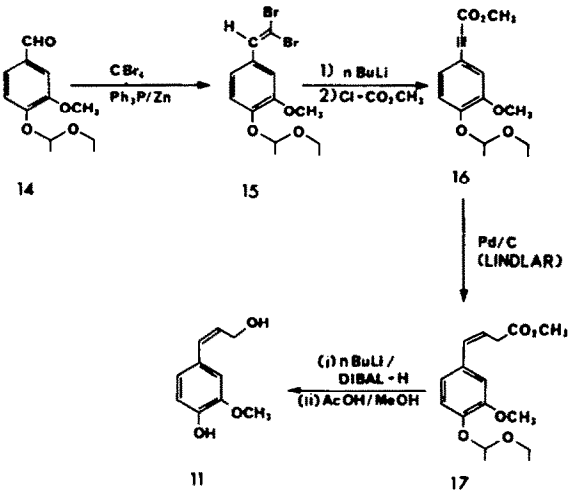
Fig. 1. Possible coniferyl alcohol end-group bonding patterns in coniferous lignin.



7, $R_1, R_2 = H$
8, $R_1 = OCH_3, R_2 = H$
9, $R_1, R_2 = OCH_3$

10, $R_1, R_2 = H$
11, $R_1 = OCH_3, R_2 = H$
12, $R_1, R_2 = OCH_3$

Scheme 1. Concept of lignification using either *cis*- or *trans*-monolignols.



14 15 16 11 17

Scheme 2. Synthesis of *cis*-coniferyl alcohol (11).

this ester with the ATE-complex [23] formed from di-isobutyl aluminium hydride and *n*-butyl lithium in toluene, followed by deprotection with acetic acid in methanol gave *cis*-coniferyl alcohol (**11**). The synthetic product (overall yield 27% from vanillin) was identical to the natural substance in every respect.

DHP lignin was synthesized, from alcohols **8** or **11**, using an H_2O_2/peroxidase enzyme system [24]. Subsequent thermal treatment of either DHP lignin with THF–water afforded only *trans*-coniferyl alcohol (**8**) in agreement with our previous observations with pre-extracted bark. These results suggest that beech bark lignin could arise via the dehydrogenative polymerization of the *cis*-monolignols **10–12**, and not from the corresponding *trans*-isomers **7–9**, assuming that the enzyme involved in lignification is a peroxidase with a low specificity for substrate, i.e. either *cis*- or *trans*-monomers are acceptable.

An alternative explanation could be that the *cis*-monolignols arise enzymatically or photochemically in bark, and, as they are not suitable substrates for lignin formation, they accumulate. It would follow therefore that the enzyme(s) involved in polymerization is (are) highly specific and that peroxidase is not the enzyme which functions *in vivo*. At the moment, it is not possible to determine which hypothesis is correct.

Finally, other plant species, e.g. bamboo (*Bambusa tulda gramineae*), and bark from the angiosperms, poplar (*Populus tremuloides Michx.*) and maple (*Acer saccharum marsh*), and the gymnosperm, black spruce (*Picea mariana*) were also analysed for their monolignol contents. Surprisingly, we were unable to detect either *cis*- or *trans*-monolignols in the hexane and methanol extracts from these plant species. Thermolysis with THF–water as before, e.g. with bamboo and poplar, though, resulted in the formation of the *trans*-isomers as expected. Additional experiments are currently underway to verify whether *cis* monolignols are involved in the biosynthesis of lignin in grasses and bark.

EXPERIMENTAL

General. HPLC of extracts was carried out using a Waters novapak C-18 column (5 μm particle size) eluted with MeCN–H_2O (3:22) at a flow rate of 0.8 ml/min. Dibal-H was obtained from Aldrich Chemical Co.

cis-Coniferyl alcohol (**11**) *and cis-sinapyl alcohol* (**12**) *from beech bark.* Fresh beech bark, removed from a 20 year old tree, was immediately sliced into narrow strips. The strips were then frozen (liquid N_2) and disintegrated in frozen form by mechanical rupture (Waring blender). The powdered bark was then removed, thawed and allowed to dry overnight under subdued light (moisture content, 6%). Sequential extraction of bark (117.4 g dry wt) in a Soxhlet afforded, after solvent removal, hexane (2.78 g, 2.37%), MeOH (10.8 g, 9.2%) and H_2O (3.26 g, 2.8%) solubles. An aliquot (~ 100 mg) was then removed for HPLC analysis.

The crude MeOH extract (1 g) was taken and applied to a silica gel column (9 cm × 10 mm) and carefully eluted with petrol–EtOAc (2:1). Selected fractions were combined and evapd to give as colourless oils, *cis*-coniferyl alcohol (**11**, 8 mg, 0.8%) and *cis*-sinapyl alcohol (**12**, 13 mg, 1.3%). Compound **11**, recrystallized from petrol–Et_2O, mp 104–106° (lit. mp [14] 104–106°); ^{1}H NMR ($CDCl_3$): δ1.53 (1H, *t*, J ~ 7 Hz, $CH_2\underline{OH}$), 3.89 (3H, *s*, OMe), 4.44 (2H, *t*, J ~ 7 Hz, $C\underline{H}_2OH$), 5.66 (1H, *s*, ArOH), 5.77 (1H, *td*, J_1 ~ 12 Hz, J_2 ~ 7 Hz, $C=C\underline{H}-CH_2OH$),

6.49 (1H, *d*, $J \sim 12$ Hz, CH=C), 6.72 (1H, *dd*, $J_1 \sim 8$ Hz, $J_2 \sim 2$ Hz, ArH), 6.75 (1H, *d*, $J \sim 2$ Hz, ArH), 6.88 (1H, *d*, $J \sim 8$ Hz, ArH); IR ν_{max}^{KBr} cm^{-1}: 3450, 3160, 1620, 1605; UV λ_{max}^{EtOH} nm (log ε): 214 (4.11), 260 (3.98), 292 (3.55); MS *m/z*: 180, 162 $[M-H_2O]^+$, 147 $[M-H_2O, Me]^+$. Calc. for $C_{10}H_{12}O_3$: 180.0786. Found: 180.0724. Compound **12**. ^1H NMR ($CDCl_3$): δ1.57 (1H, *m*, $CH_2\underline{OH}$), 3.89 (3H, *s*, OMe), 4.44 (2H, *dd*, $J_1 \sim 8$ Hz, $J_2 \sim 2$ Hz, $C\underline{H}_2OH$), 5.55 (1H, *s*, ArOH), 5.81 (1H, *td*, $J_1 \sim 14$ Hz, $J_2 \sim 8$ Hz, C=C$\underline{H}$-CH_2OH), 6.48 (2H, *s*, ArH), 6.51 (1H, *d*, $J \sim 14$ Hz, $CH=\underline{C}$); MS *m/z*: 210 $[M]^+$, 182, 167, 149. Calc. for $C_{11}H_{14}O_4$: 210.0892. Found: 210.0949.

trans-*Coniferyl* (**8**) *and sinapyl* (**9**) *alcohols from pre-extracted beech bark*. Pre-extracted beech bark (59.28 g) was suspended in a soln of THF–H_2O (200 ml, 1:1) in a Teflon-lined stainless steel bomb under an atm of N_2. The bomb was then placed in an oil bath at 180° for 50 min, cooled to 20°, filtered and the THF removed *in vacuo*. The resulting aq. suspension was then extracted with Et_2O (10 × 100 ml). The Et_2O extracts were then combined, dried (Na_2SO_4) and evapd to dryness (1.66 g). Flash chromatography on silica gel as before afforded *trans*-coniferyl (**8**, 15 mg) and *trans*-sinapyl (**9**, 12 mg) alcohols.

cis/trans-*Coniferyl* (**11**, **8**) *and sinapyl* (**12**, **9**) *alcohols from unextracted beech bark*. Unextracted beech bark (47 g) was thermally treated as above in a soln of THF–H_2O to afford Et_2O solubles (2.5 g). The Et_2O extract (1 g) was applied to a silica gel column and eluted as before with petrol–EtOAc (2:1) to give *trans*-coniferyl (**8**, 10 mg), *cis*-coniferyl (**11**, 35 mg), *trans*-sinapyl (**9**, 10 mg) and *cis*-sinapyl (**12**, 41 mg) alcohols.

trans-*Coniferyl* (**8**) *and sinapyl* (**9**) *alcohols from beech wood*. Unextracted beech wood (23.78 g) was suspended in a soln of THF–H_2O as before to afford, after chromatography *trans*-coniferyl (50 mg) and *trans*-sinapyl (25 mg) alcohols.

trans-*Coniferyl alcohol* (**8**) *from bamboo*. Unextracted bamboo wood (15 g dry wt) was treated with THF–H_2O as before. Chromatography of the Et_2O soluble fraction (1.3 g) afforded *trans*-coniferyl alcohol (**8**, 6 mg).

trans-*Coniferyl alcohol* (**8**) *from poplar*. Poplar wood (50 g dry wt) was extracted with hexane, MeOH and H_2O. The resulting extracted material was immersed in THF–H_2O as before. Chromatography of the Et_2O soluble fraction (1.06 g) afforded *trans*-coniferyl alcohol (**8**, 30 mg).

Dehydrogenative polymerized lignin (*DHP*) *from cis-coniferyl alcohol* (**11**). Peroxidase enzyme (4.1 mg, activity 860 units, Boehringer Mannheim) was added to a soln of degassed 0.025 M Na_2HPO_4–NaOH buffer (150 ml) at pH 7.5 under an atm of N_2. Solns of H_2O_2 (122 μl of a 11.8 M H_2O_2 soln dissolved in 0.025 M Na_2HPO_4–NaOH buffer, 300 ml, 1.44 mmol H_2O_2, pH 7.4) and *cis*-coniferyl alcohol (**11**, 259 mg, 1.44 mmol in 0.025 M Na_2HPO_4–NaOH, 300 ml) were then added, at equal rates, to the peroxidase soln over a period of 43 hr. The resulting cloudy, pinkish, orange suspension was then centrifuged for 3 hr at 5500 rpm. The ppt so obtained was resuspended and centrifuged for an additional 2.5 hr at 8000 rpm. The solid material was then freeze-dried to give the DHP polymer as a pink–grey solid (133 mg, 52%).

trans-*Coniferyl alcohol* (**8**) *from DHP polymer*. DHP polymer (60 mg) was suspended in a soln of THF–H_2O (5 ml) and autoclaved as described above. The Et_2O solubles were then removed by extraction. Analysis by HPLC showed only the presence of the *trans*-isomer **8**, which was subsequently collected by HPLC (3 mg).

3-Methoxy-4-(ethoxyethylidene-oxy)-benzaldehyde (**14**). A mixture of vanillin (5 g, 32.86 mmol), ethyl vinyl ether (4 ml, 41.8 mmol) and pyridinium *p*-toluenesulphonate (300 mg) in dry CH_2Cl_2 (100 ml) was stirred for 6 hr at room temp. Aq. $NaHCO_3$ (20 ml, 5% soln) was added and the mixture stirred for 10 min. The CH_2Cl_2 layer was separated, washed with brine (20 ml), dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude product was applied to a silica gel column and then carefully eluted with petrol–EtOAc (4:1) by flash CC. The ethoxyethylidene derivative **14** was obtained as a solid (6.6 g, 90%), mp 34–36°; ^1H NMR ($CDCl_3$): δ1.22 (3H, *t*, $J = 7.0$ Hz, Me), 1.58 (3H, *d*, $J = 6$ Hz, Me), 3.63 (2H, *q*, $J = 7.0$ Hz, CH_2), 3.92 (3H, *s*, OMe), 5.50 (1H, *q*, $J = 6.0$ Hz, C$\underline{H}$Me), 7.15 (1H, $J = 9.0$ Hz, ArH), 7.37 (1H, *dd*, $J_1 = 9.0$ Hz, $J_2 = 2.0$ Hz, ArH), 7.38 (1H, *d*, $J = 2.0$ Hz, ArH) and 9.80 (1H, *s*, CHO); MS *m/z*: 224 $[M]^+$, 153, 152 $[M-$ethoxyethylidene group$]^+$, 151, 137 $[M-$ethoxyethylidene group, $-Me]^+$.

1,1-Dibromo-2-(3-methoxy-4-ethoxyethylideneoxyphenyl)-ethylene (**15**). A soln of CBr_4 (21.4 g, 64.5 mmol) in dry CH_2Cl_2 (50 ml) was added dropwise, over a period of 20 min, to a suspension of Zn dust (4.22 g, 64.5 mmol) and triphenyl phosphine (16.91 g, 64.5 mmol) in dry CH_2Cl_2 (75 ml) at 20°. The mixture was stirred for 2 hr, after which the protected vanillin derivative **14** (6.24 g, 27.86 mmol) in CH_2Cl_2 (60 ml) was then added. Following stirring overnight at room temp., the suspension was filtered and the filtrate concd *in vacuo* to *ca* 3–5 ml. The crude reaction mixture concentrate was then applied to a column of silica gel. Elution with petrol–EtOAc (4:1) gave a mixture of the protected dibromo-olefin **15** and its de-protected analogue, free from triphenylphosphine oxide. The olefinic mixture was then dissolved in dry CH_2Cl_2 (75 ml) containing ethyl vinyl ether (3.5 ml, 36.6 mmol) and pyridinium tosylate (100 mg), and stirred at room temp. for 30 min. In a manner analogous to **14**, the pure dibromo-olefin **15** was obtained as a yellow oil (7.94 g, 75%); ^1H NMR ($CDCl_3$): δ1.20 (3H, *t*, $J = 7.0$ Hz, Me), 1.50 (3H, *d*, $J = 6$ Hz, Me), 3.60 (2H, *dq*, $J_1 = 7$ Hz, $J_2 = 2$ Hz, $C\underline{H}_2$Me), 3.82 (3H, *s*, OMe), 5.33 (1H, *q*, $J = 6$ Hz, C$\underline{H}$Me) and 6.97–7.3 (3H, *m*, ArH).

1-Carbomethoxy-2-(3-methoxy-4-ethoxyethylideneoxyphenyl)-acetylene (**16**). *n*-BuLi (12.3 ml, 1.6 M in hexane, 19.68 mmol) was slowly added dropwise to a soln of dibromo-olefin (**15**, 3 g, 7.89 mmol) in dry THF (30 ml) at −78° under N_2. Following stirring at this temp. for 30 min, a soln of methyl chloroformate (900 mg, 9.52 mmol) in dry THF (20 ml) was added. The suspension was then stirred for 1 hr, after which the temp. was raised to 20° and stirring maintained for an additional 1 hr. H_2O (~ 3 ml) was then carefully added and the THF removed *in vacuo*. H_2O (20 ml) was added to the residue, which was then extracted with Et_2O (2 × 75 ml). The organic solubles were then combined, washed with brine (20 ml), dried ($MgSO_4$) and evapd. The crude product was applied to a silica gel column and carefully eluted with petrol–EtOAc (4:1) by flash CC. Fractions containing the acetylenic ester **16** were combined to give the colourless oil (1.16 g, 53%); ^1H NMR ($CDCl_3$): δ1.18 (3H, *t*, $J = 7$ Hz, Me), 1.52 (3H, *d*, $J = 6$ Hz, Me), 3.60 (2H, *q*, $J = 7$ Hz, $C\underline{H}_2$Me), 3.80 (3H, *s*, OMe), 3.83 (3H, *s*, OMe), 5.38 (1H, *q*, $J = 6$ Hz, C$\underline{H}$Me), 7.03 (3H, *m*, ArH); IR ν_{max}^{neat} cm^{-1}: 2215, 1715; MS *m/z*: 278 $[M]^+$, 206 $[M-$ethoxyethylidene group$]^+$, 175 $[M-$ethoxyethylidene group $-$OMe$]^+$. Calc. for $C_{15}H_{18}O_5$: 278.1154. Found: 278.1175.

Methyl 3-(3-Methoxy-4-ethoxyethylideneoxyphenyl) prop-2Z-enoate (**17**). To a soln of acetylenic ester (**16**, 2.39 g, 8.6 mmol) in 95% EtOH (8 ml) was added freshly prepared Lindlar catalyst (1 g)[22]. Following consumption of 1 eq of H_2, the catalyst was removed by filtration and the resulting filtrate evapd to give the *cis*-olefinic ester **17** as a colourless oil (2.31 g, 96%); ^1H NMR ($CDCl_3$): δ1.20 (3H, *t*, $J = 7$ Hz, Me), 1.53 (3H, *d*, $J = 6$ Hz, Me), 3.50 (2H, *q*, $J = 7$ Hz, $C\underline{H}_2$Me), 3.60 (3H, *s*, OMe), 3.75 (3H, *s*, OMe), 5.23 (1H, *q*, $J = 6$ Hz, C$\underline{H}$Me), 5.65 (1H, *d*, $J = 13$ Hz, CH=C), 6.58 (1H, *d*, $J = 13$ Hz, C=CH), 6.95 (2H, *m*, ArH), 7.45 (1H, *br d*, ArH); IR ν_{max}^{neat} cm^{-1}: 1725, 1635, 1605; MS *m/z*: 280

$[M]^+$, 209, 208 [M − ethyl vinyl ether]$^+$, 206, 193, 178, 177.

3-(3-Methoxy-4-hydroxyphenyl)-prop-2Z-enol (**11**). 'ATE' complex was prepared by mixing DIBAL-H (15 ml, 1.5 M in toluene, 22.5 mmol) and toluene (23.5 ml) at room temp. The soln was cooled to 0° and *n*-BuLi (14.06 ml, 1.6 M in hexane, 22.5 mmol) was then added. The mixture was stirred at 0° for 30 min before use. 'ATE' complex was then added to *cis*-olefinic ester **17** (2 g, 7.14 mmol) in dry THF (25 ml) under N_2 at −78°. Following stirring at this temp. for 30 min, the temp. was raised to 20° and stirring maintained for an additional 1 hr. MeOH (2 ml) was then carefully added and the solvent removed *in vacuo*. The residue was taken up in ice-H_2O (50 ml), acidified to pH 5 with HCl (0.3 M) and extracted with Et_2O (3 × 100 ml). The extracts were then combined, washed with 5% $NaHCO_3$ soln (2 × 20 ml), brine (2 × 20 ml), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to give a mixture of *cis*-coniferyl alcohol (**11**) and its ethoxyethylidene derivative. The protecting group was then removed by dissolving the crude product in MeOH (65 ml), adding HOAc (1 ml) and stirring at room temp. for 6 hr under N_2. Solid $NaHCO_3$ (1 g) was added and the solvent removed *in vacuo*. To the residue was added H_2O (20 ml) and the whole extracted with Et_2O (2 × 75 ml). The Et_2O extracts were combined, washed with brine (20 ml) and dried ($MgSO_4$). The crude product was applied to a silica gel column and carefully eluted with petrol–EtOAc (2:1). Fractions containing *cis*-coniferyl alcohol (**11**) were combined and evapd. Recrystallization from petrol–EtOAc afforded **11** (1.0 g, 78%).

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