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A Biomimetic Route to Lignin Model Compounds *via* Silver (I) Oxide Oxidation

1. Synthesis of Dilignols and Non-cyclic Benzyl Aryl Ethers

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Keywords

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α-O-4-Bonds
α-O-γ Bonds
β-O-4 Quinone methide
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NMR
DHP

Summary

The oxidative coupling of (*E*)-coniferyl alcohol using 1.5 equiv. of silver (I) oxide in methylene chloride led to the formation of β-5-coupled dehydrodiconiferyl alcohol in 50% yield, whereas in 1 : 1 methylene chloride: water solution, the β-β-coupled dehydrodimer pinoresinol was obtained as the major reaction product in 25% yield. In aqueous (pH 5–8) and non-aqueous solutions, the quinone methide generated by β-O-4 coniferyl alcohol radical coupling rapidly led to the formation of significant amounts of the non-cyclic α-O-4 benzyl aryl ethers; *erythro*-guaiacylglycerol-α-dehydrodiconiferyl-β-coniferyl-bis-ether, *erythro*-guaiacylglycerol-α,β-bis-coniferyl ether and *erythro*-guaiacylglycerol-α-pinoresinol-β-coniferyl-bis-ether, *via* phenol addition. The addition of water became predominant only under acid catalysis. Non-cyclic α-O-γ benzyl alkyl ethers were not obtained. These findings, together with literature precedents, have implications for lignin biosynthesis.

Introduction

The key mechanism in lignin biosynthesis is the oxidative coupling of phenols (Harkin 1967; Freudenberg 1968; Sarkanen 1971). Delocalised phenoxyl radicals, generated upon phenol dehydrogenation of *p*-hydroxycinnamyl alcohol monomers and intermediate lignin units, couple in a variety of ways to build up the lignin polymer. This free radical-mediated dehydrogenative polymerization is thought to be initiated by peroxidases and/or laccases (reviewed in Dean and Eriksson 1992). Peroxidases, in the presence of hydrogen peroxide (H_2O_2), oxidize phenolic substrates to phenoxyl radicals *via* a two-step one-electron transfer catalytic cycle (Dunford 1982). This peroxidases-H_2O_2 system is commonly used for the preparation of synthetic lignins, or dehydrogenation polymers (DHPs).

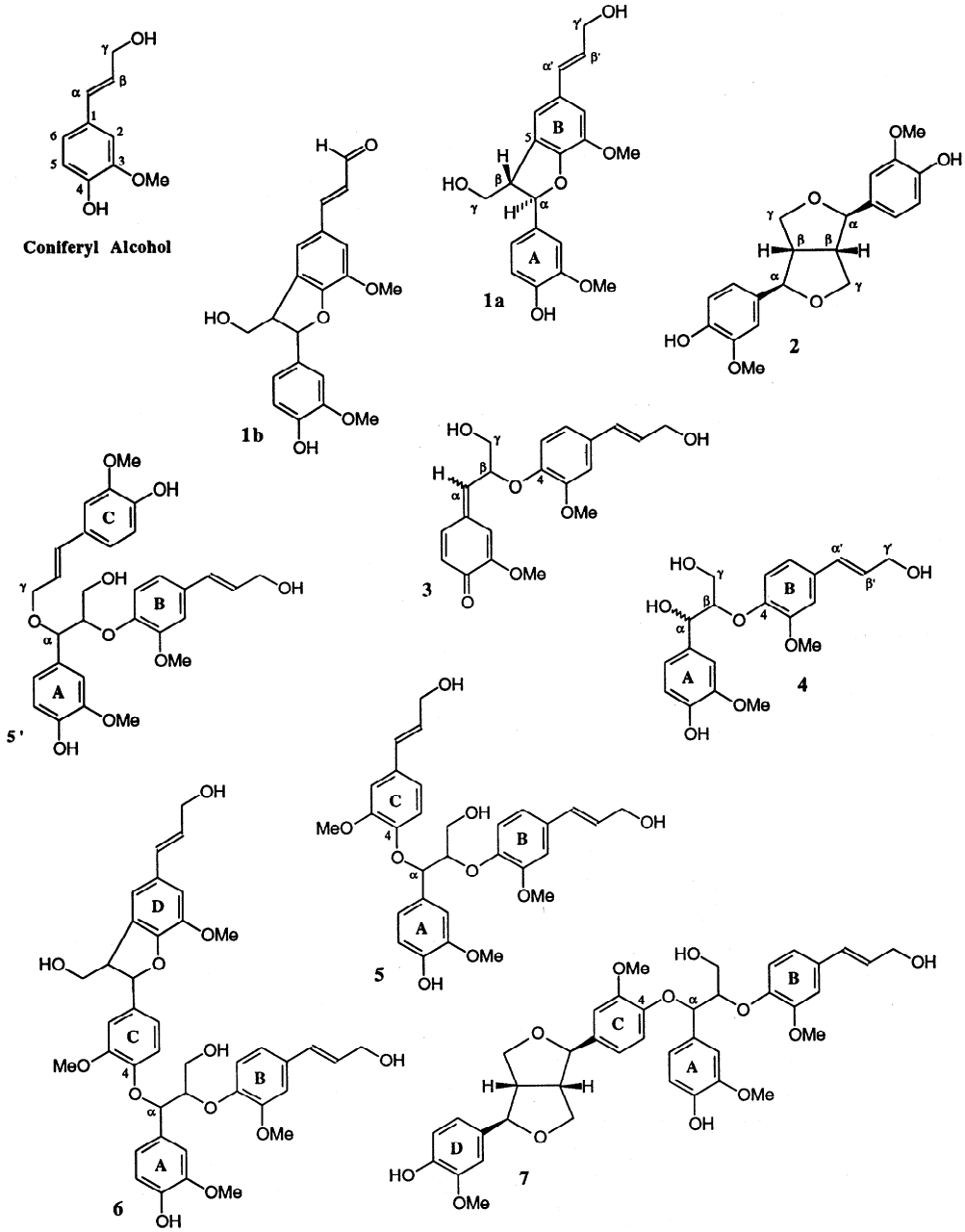
These DHPs, as high molecular mass lignin model compounds, have been used to investigate the chemical bonds between the *p*-hydroxyphenylpropanoid structural units in lignins and provide a powerful tool to determine the chemical pathways available during lignification processes. Elucidation of the DHP and lignin structures requires the synthesis and characterization of adequate low molecular mass model compounds and strongly relies on the application of NMR spectroscopic techniques (Lüdemann and Nimz 1974a, b; Nimz *et al.* 1974; Nimz and Lüdemann 1976; Gagnaire and Robert 1977; Lundquist 1979; Brunow and Lundquist 1980; Nimz *et al.* 1981, 1984; Ralph *et al.* 1992a, b, 1993).

In the past, the oxidative coupling of *p*-propenyl phenol derivatives using one-electron oxidants such as ferric chloride, potassium ferricyanide, or peroxidases-H_2O_2, provided access to dimeric lignin model compounds (Leopold 1950; Sarkanen and Wallis 1973; Wallis 1973; Katayama and Fukuzumi 1978), with more or less preparative efficiency. High-yielding synthetic schemes for the preparation of aryl-glycerol-β-aryl ethers, which model the prominent β-O-4 interunit linkage in lignin isolates, are in common use (Nakatsubo *et al.* 1975; Nakatsubo and Higuchi 1980; Landucci *et al.* 1981; Ralph and Helm 1991). Phenylcoumarans such as β-5-coupled dehydrodiconiferyl alcohol (**1a**) have also been the targets of a few synthetic reports, but the schemes proposed remain particularly tedious and low-yielding (Nakat-

subo and Higuchi 1979; Brunow and Lundquist 1984). Improved access to multi-gram quantities of coniferyl alcohol (Quideau and Ralph 1992) led us to look for a more expedient route to the β-5-coupled dehydrodimer **1a** that we needed for derivatization with *p*-hydroxycinnamic acids in the framework of our investigations on the *p*-hydroxycinnamic acid/lignin complex in grass cell walls (Ralph *et al.* 1993). An oxidative coupling approach was chosen using silver (I) oxide as a one-electron oxidant, and it was found that variation of the reaction conditions provided a biomimetic route to additional lignin model compounds. This report describes convenient methods for relatively selective preparations of phenylcoumaran and pinoresinol models, and examines the *in situ* trapping of β-O-4 quinone methides to yield benzyl alcohols and/or non-cyclic α-O-4 linked benzyl aryl ethers. The complete NMR characterization of the trimeric and tetrameric non-cyclic benzyl aryl ethers, potentially important branching units in lignins, is reported in the accompanying paper (Quideau and Ralph 1994).

Results and Discussion

Silver (I) oxide (Ag_2O) oxidation was first used for the synthesis of lignin model compounds by Zanarotti (1983). Oxidation of propenylphenols, such as (*E*)-isoeugenol, in benzene generated a β-O-4-type quinone methide which, upon acid-catalysed hydration, led to the formation of the corresponding benzyl



alcohol, isolated in 75% yield. Hawkes *et al.* (1986) obtained only 56% of the β-O-4-type benzyl alcohol, together with 18% of the β-5-coupled dehydrodiisoeugenol, by using Zanarotti's method. This somewhat selective Ag_2O-mediated C–O coupling is noteworthy since the oxidative coupling of (*E*)-isoeugenol utilizing ferric chloride, laccase-O_2 or peroxidases-H_2O_2 as the oxidizing system gives rise to the predominant formation of C–C coupled products, i.e. β-5- and β-β-coupled dehydrodimers (Leopold 1950; Sarkanen and Wallis 1973). Interestingly, β-O-4 dehydrodimers were predominantly obtained from the peroxidases-H_2O_2 oxidation of (*Z*)-isoeugenol (Sarkanen and Wallis 1973). Charge-transfer complex intermediates were proposed to rationalize this selectivity change in the radical coupling mode of the two isomers. On the other hand, Zanarotti (1983) suggested a coordination effect of silver (I) cations to explain the high selectivity of the β-O-4 coupling mode with (*E*)-isoeugenol. In our case, the oxidation of the lignin monomer (*E*)-coniferyl alcohol (which differs from (*E*)-isoeugenol by the presence of an hydroxyl group at the side-chain γ-position) in methylene chloride or acetone with 1.5 equiv. of Ag_2O, gave β-5-coupled dehydrodiconiferyl alcohol (**1a**) in yields up to 50% (Table 1). The use of methylene chloride as solvent allowed the best reproducibility and yield. This one-step synthesis of **1a** constitutes an improved oxidative coupling method for the preparation of this important dilignol and a convenient alternative to the previously reported multistep synthesis (Nakatsubo and Higuchi 1979). To our knowledge, the best oxidative coupling yield previously reported for **1a** is 26% (Freudenberg and Schlüter 1955). The *trans*-configuration of the 2,3-dihydrobenzofuran moiety in **1a** is assumed from previously reported stereochemical determination of related phenylcoumarans (Aulin-Erdtman and Tomita 1963; Nakatsubo and Higuchi 1975a; Hawkes *et al.* 1986). Besides traces of the aldehydic phenylcoumaran **1b**, coniferaldehyde and small amounts of the β-β-coupled dehydrodimer **2** (pinoresinol), the other main reaction product was a polymeric material. The yellow color of the reaction mixture, characteristic of the presence of quinone methide intermediates, remained throughout the allowed reaction time (see Experimental and Table 1, conditions A and B). Removal of the reaction solvent presumably caused the formation of an α-O-4 ether polymer, *via* polymerization of the β-O-4 quinone methide intermediate **3** (Freudenberg and Werner 1964; Ralph 1989). About 5–10% of *erythro*-guaiacylglycerol-α-dehydrodiconiferyl-β-coniferyl-bis-ether (**6**), formed *via* phenol addition of the dilignol **1a** to the β-O-4 quinone methide **3**, was also isolated (Table 1). In d_6-acetone solvent, monitoring of the reaction by ^{1}H-NMR analysis confirmed the *in situ* formation of this non-cyclic α-O-4 linked benzyl aryl ether (**6**). Decreasing the reaction time increased the proportion of **6** and concomitantly decreased the proportion of **1a** and polymeric material, indicating that addition of **1a** to the quinone methide **3** is kinetically controlled. When the reaction was quenched with HCl until decoloration of the yellow solution (Table 1, condition C), substantial amounts of the benzyl alcohol **4** (32%), formed by acid-catalyzed addition of water to **3**, were isolated as an approximately 50 : 50 *erythro : threo* mixture. This result confirms the nature of the polymeric material mentioned above and indicates that this Ag_2O-mediated free radical coupling of coniferyl alcohol is limited to the formation of dehydrodimers. Compound **4** could also have been partially formed following hydrolysis of varying amounts of **6**. The α-O-4 linked tetralignol **6** and trilignol **5** (guaiacylglycerol-α,β-bis-coniferyl ether, see below) were first obtained by Freudenberg and Friedmann (1960) by oxidation of coniferyl alcohol with manganese dioxide in acetone.

On the formation of non-cyclic benzyl aryl ethers

The occurrence of non-cyclic α-O-4 benzyl aryl ether linkages in lignin has been a controversial subject in the literature (Leary 1980, 1982; Nimz 1981; Nimz *et al.* 1984). Leary has refuted the

Table 1. Silver (I) oxide oxidations of coniferyl alcohol

Conditions	Reaction time	Isolated compounds (yield %)					
		1a	**2**	**4**	**5**	**6**	**7**
A	24h	50	4	–	traces	6	–
B	10h	44	5	–	traces	8	–
C	10h	47	5	32	–	traces	–
D	4h	24	14	18	–	–	–
E	5h	24	13	traces	24	5	traces
F	30min	17	10	traces	14	15	7
G	1h	12	25	traces	10	10	traces

A: dry methylene chloride; B: dry acetone; C: dry acetone + 1M HCl quenching; D: 1 : 2 acetone : pH2.5 citrate buffer (pH3.1); E: 1 : 2 acetone : pH4.3 citrate buffer (pH5.3); F: 1 : 1 acetone : water (pH7.4); G: 1 : 1 methylene chloride : water (pH ~ 8).

possibility of such bonds on the basis of the observation that addition of vanillyl alcohol to its derived quinone methide occurred exclusively via the primary hydroxyl group in neutral or weakly acidic aqueous solutions (Hemmingson and Leary 1980). This led to the proposal that the formation of non-cyclic α-O-γ benzyl alkyl ethers is favored over the formation of non-cyclic α-O-4 benzyl aryl ethers during lignification (Leary 1980). Structure **5′** is shown as an example of an α-O-γ linked benzyl alkyl

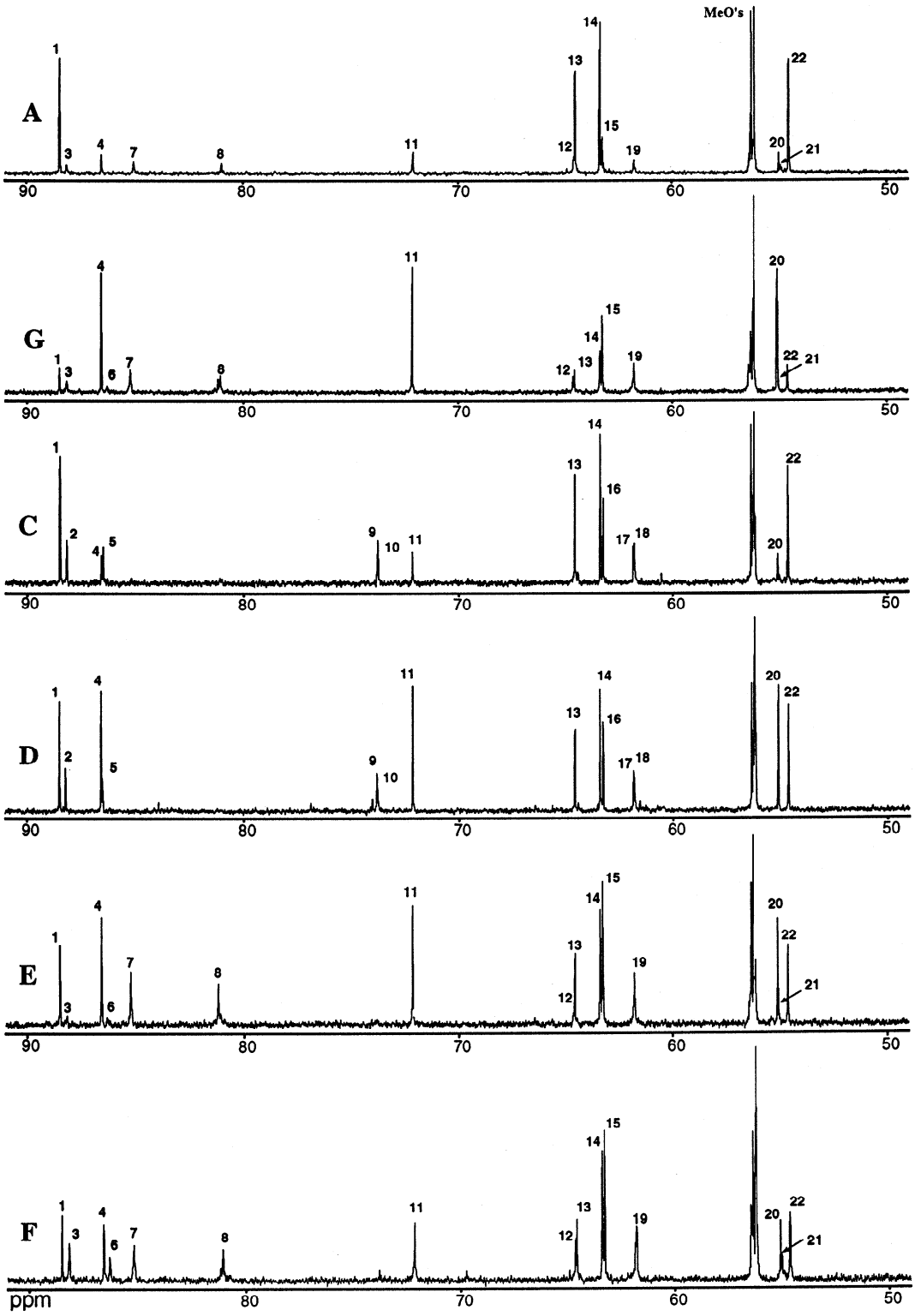


Fig. 1. Aliphatic regions of ^{13}C NMR spectra of product mixtures of silver (I) oxide oxidations of coniferyl alcohol showing product ratios prior to isolation; see Table 1 and text for isolated compound yields and experimental details. Signal assignments are given in Table 2.

Table 2. Chemical shifts and assignment of signals in aliphatic regions of ^{13}C NMR spectra of product mixtures of silver (I) oxide oxidations of coniferyl alcohol[a,b]

Signal #	Chemical shifts	Assignments
1	88.48	C-α (**1a**)
2	88.21	C-β (*threo*-**4**)
3	88.15	C-α (**6**, unit C)
4	86.56	C-α (**2**), C-α (**7**, unit D)
5	86.49	C-β (*erythro*-**4**)
6	86.35	C-α (**7**, unit C)
7	85.19–85.38[c]	C-β (**5**, **6** and **7**, unit A)
8	81.08–81.21[c]	C-α (**5**, **6** and **7**, unit A)
9	73.82	C-α (*threo*-**4**)
10	73.76	C-α (*erythro*-**4**)
11	72.20	C-γ (**2**), C-γ (**7**, unit C and D)
12	64.63	C-γ (**6**, unit C)
13	64.55	C-γ (**1a**)
14	63.38	C-γ′ (**1a**), C-γ (**6**, unit D)
15	63.27	C-γ (**5**, **6** and **7**, unit B), C-γ (**5**, unit C)
16	63.25	C-γ′ (*threo*-**4** and *erythro*-**4**)
17	61.83	C-γ (*threo*-**4**)
18	61.79	C-γ (*erythro*-**4**)
19	61.77–61.82[c]	C-γ (**5**, **6** and **7**, unit A)
20	55.15	C-β (**2**), C-β (**7**, unit C and D)
21	55.02	C-β (**6**, unit C)
22	54.63	C-β (**1a**)

[a] Chemical shifts are given in ppm (acetone-d_6); the erythro/threo C-γ and C-α chemical shift assignment can be interchanged. [b] E.g. unit A refers to the phenylpropanoid unit with aromatic ring A; see figures for atom and aromatic ring designations. [c] See Table 3.

ether which would be formed upon addition of the primary hydroxyl group of coniferyl alcohol to the quinone methide **3**. Leary's suggestion has been recently supported by the results of Shevchenko *et al.* (1992) on the addition of *p*-hydroxybenzyl alcohols and coniferyl alcohol, in neutral chloroform solution, to quinone methides derived from guaiacyl propanol and β-aryl ether model compounds (1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)ethane/propan-1-ol). Benzyl alkyl ethers were the only products formed upon nucleophilic addition to these quinone methides. However, using a quinone methide derived from a lignin model which possesses the hydroxymethyl group (1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-propan-1,3-diol), Brunow *et al.* (1989) observed that the addition of vanillyl alcohol occurred exclusively via the phenolic hydroxyl group in neutral aqueous and non-aqueous solutions.

In order to clarify these contradictory observations on the reactivity of quinone methide intermediates during lignification, we were interested in effecting the silver (I) oxide oxidation of coniferyl alcohol in aqueous solutions, at different pHs. The main advantages of this biomimetic model system are the *in situ* generation of genuine lignin quinone methide intermediates *via* β-O-4 coupling of coniferyl alcohol radicals, the presence of water, phenols and alcohols for competing nucleophilic addition to β-O-4 quinone methides, and the simultaneous formation of typical lignin dehydrodimers, such as **1a** and **2**. The diagnostic aliphatic regions of the ^{13}C-NMR spectra of the different product mixtures prior to isolation are displayed in Figure 1 and the yields of the isolated major products are given in Table 1. Signal assignments are given in Table 2. When the reaction was carried out in a 1 : 2 acetone : pH2.5 citrate buffer solution (the pH value of the reaction mixture determined by pH-meter was 3.1, see Experimental and Table 1, condition D), significant amounts (18%) of the benzyl alcohol **4** were isolated as a 40 : 60 *erythro* : *threo* mixture. This indicates that, under these acidic conditions, water is the preferred nucleophile attacking the more reactive carbonium ion obtained by protonation of the quinone methide **3**. This is also supported by the highest yield of **4** (32%) obtained when HCl quenching of the dry acetone reaction solution was used (Table 1, condition C). Although the overall yield of isolated products under condition D was relatively low (56%), no non-cyclic benzyl aryl or alkyl ethers were detected by ^{1}H and ^{13}C NMR analysis.

At pH 4.3–5.3 (Table 1, condition E), only traces of **4** were observed by ^{1}H NMR analysis, whereas significant amounts of the *erythro* isomers of the non-cyclic benzyl aryl ethers **5** (24%) and **6** (5%) were isolated. The benzyl aryl ethers **5** and **6** were found to be stable under these conditions and no isomerisation to *erythro* : *threo* mixtures was observed by using longer reaction times. In this heterogeneous Ag_2O-solvent system, the addition of the phenolic hydroxyl of unoxidized coniferyl alcohol to the quinone methide **3** to give **5** efficiently competes with the addition of water. Non benzyl citrate adducts from the possible trapping of **3** by the buffer citric acid were detected under these slightly acidic conditions.

It is noteworthy that the formation of pinoresinol (**2**) was more important in aqueous solutions than in anhydrous organic solvents. This furofuranoid compound was obtained in a reproducible yield of 25% (the highest oxidative coupling yield so far reported for **2**) when the reaction was performed in a two-phase 1 : 1 methylene chloride : water solution (Table 1, condition G), providing again a convenient one-step route to an important lignin model compound (Fujimoto *et al.* 1982). The specific reason for these changes in the selectivity of the coniferyl alcohol radical coupling mode is not understood, but the polarity of the reaction solvent appears to have a substantial effect. The β-β coupling is apparently more important in polar aqueous solvents, whereas the β-5 and β-O-4 coupling predominates in less

polar or non-polar organic solvents. Similar electronic effects due to the use of solvents of differing polarity have previously been noted in the coupling of 3,5-disubstituted *p*-hydroxycinnamyl alcohols (Tanahashi *et al.* 1976).

Under neutral conditions (pH 7.4), in 1 : 2 acetone : water solutions, the reaction rate was drastically increased and the complete disappearance of coniferyl alcohol was noted after *ca.* 30 min. by TLC monitoring (Table 1, condition F). Besides dehydrodiconiferyl alcohol (**1a**) and pinoresinol (**2**), the *erythro* isomer of the benzyl aryl ethers **5, 6** and **7** (guaiacylglycerol-α-pinoresinol-β-coniferyl-bis-ether) were isolated. The formation of the tetralignol **7** has not previously been reported. According to NMR analysis, the polymeric fraction mainly consisted of α-O-4 ether polymers, formed by polymerization of β-O-4 quinone methides upon solvent removal. This result suggests relatively high stability of the β-O-4 quinone methides, even under neutral aqueous conditions. In contrast, Nakatsubo *et al.* (1976) have reported the rapid and quantitative formation of benzyl alcohol when a β-O-4 quinone methide solution was added to water, and peroxidase-catalyzed dehydrogenation of coniferyl alcohol has been reported to give the benzyl alcohol **4** in yields up to 20% (Katayama and Fukuzumi 1978). In our case, only traces of **4** were obtained under neutral conditions. When the Celite-filtered reaction mixture was left stirring at ambient temperature for several days, a partial degradation of the benzyl aryl ether compounds (**5–7**) gave rise to increasing amounts of **4**, as indicated by ^{1}H NMR analysis. Again, no formation of acyclic benzyl alkyl ethers were detected. These results are in excellent agreement with Brunow and Sipilä's observations on the effect of pH on the reactivity of β-O-4 quinone methides with water, phenols and alcohols (Brunow *et al.* 1989; Sipilä and Brunow 1991a, b). Increasing the reaction time resulted in an increasingly alkaline reaction mixture (up to pH ~ 10), presumably due to the formation of silver hydroxide from the reaction of silver oxide with water in this non-buffered aqueous solution. Under these basic conditions, the hydroxide ion HO^- still did not compete with the phenolates and only traces of benzyl alcohol were detected. We have previously observed that, in organic solvents, addition of hydroxide to quinone methides is an extremely poor reaction (Ralph 1989). The relative proportions of the main reaction products were similar to the ones obtained under neutral conditions. No styryl ether or stilbene derivatives, potentially formed from **3** or **1a** *via* base-catalyzed retro-aldol reaction, were isolated.

Implications

During the silver (I) oxide-mediated oxidative coupling of (*E*)-coniferyl alcohol in aqueous (pH 5–8) and non-aqueous solutions, quinone methide intermediates generated by β-O-4 radical coupling rapidly led to the formation of non-cyclic benzyl aryl ethers *via* phenol addition. These α-O-4 linked structures were the predominant products resulting from nucleophilic attack onto β-O-4 quinone methides and were relatively stable under slightly acidic to alkaline reaction conditions. No non-cyclic α-O-γ linked benzyl alkyl ethers were detected. These results are in excellent agreement with Brunow and Sipilä's investigations, but in total opposition to those of Leary and Shevchenko. It is important to note that the studies of Leary and Shevchenko centered on the preparation of quinone methides which lacked the γ-hydroxyl. This functional group thus seems to have an important effect on the regiochemical outcome of the competing phenol/alcohol nucleophilic addition to β-O-4 quinone methides, and stresses the need for caution in interpreting results from simple models. Although the non-cyclic benzyl aryl ethers slowly hydrolyzed in neutral aqueous solution, the cleavage of α-O-4 bonds did not lead to the formation of α-O-γ bonds. The addition of water, which gives rise to a β-O-4 benzyl alcohol, the most abundant substructure in lignins, became predominant under acid catalysis.

An interpretation of our results would be that, under "bulk" dehydrogenative polymerization conditions (Sarkanen 1971; Lai and Sarkanen 1975), the formation of non-cyclic benzyl aryl ethers is kinetically favored over the formation of benzyl alcohols. As usually suggested, these α-O-4 linked structures would become resistant to hydrolysis upon rapid etherification via dehydrogenation of the phenolic moiety and subsequent radical coupling. This "bulk" polymer should have high proportions of β–5-coupled structures (and β-β-coupled structures especially if sinapyl alcohol is present) and cinnamyl alcohol end-groups due to extensive dimerization *via* β-5 and β-O-4 radical coupling modes, and a high degree of branching due to the occurrence of the α-O-4 bonds. Although in contradiction with Sarkarnen's proposition (Sarkanen 1971), we assume that this type of polymer would be characteristic of lignin-rich regions such as cell corners and compound middle lamella, in which lignification initiates. This assumption is supported by the results of Eom and Meshitsuka (1987, 1989) on the chemical characterization of lignins in the differentiating xylems. This "bulk" polymer would also be characteristic of newly formed lignin in primary walls of forage plants in which *p*-hydroxycinnamoyl esters, e.g. feruloyl esters, are postulated to serve as a lignification template (Fry and Miller 1989; Ralph and Helm 1993). We have also observed the importance of the β-5 radical coupling mode in the case of the oxida-

tion of a feruloylated arabinofuranoside with silver (I) oxide (Ralph *et al.* 1992b), as well as with peroxidases-H_2O_2 (Ralph *et al.* 1992a), suggesting that β-5-coupled dehydrodiferuloyl esters are likely to occur as hemicellulose cross-linkers in unlignified forage primary cell walls, in addition to the commonly proposed 5-5-coupled dehydrodiferuloyl esters.

As lignin continues to deposit through the cell wall, an "end-wise" polymerization would become more important. In other words, because of a lower concentration of available *p*-hydroxycinnamyl alcohol monomers, a predominance of the β-O-4 radical coupling mode would occur by coupling between the more stable monomeric β-radical mesomers and the more reactive and sterically accessible oligo/polymeric phenoxyl radicals. The predominant addition of water to the resulting quinone methides could then be due to the high concentration and mobility of the water molecules, or acid-catalysis. Hydrolysis of α-O-4 bonds initially formed is also conceivable if these units are not rapidly etherified. This "end-wise" polymer, that we presume to be characteristic of secondary wall lignin, would thus have a high proportion of benzyl alcohol β-aryl ether structures and a higher linear character than a "bulk" polymer. Higher content of non-cyclic α-O-4 bonds in middle lamella than in secondary wall lignins have indeed been reported (Sorvari *et al.* 1986). Assuming that a "bulk" polymerization should allow the best conditions for the formation of non-cyclic α-O-γ linked lignin substructures, their occurrence is likely to be minimal based on our observations and those of others. This is not to say that lignin-carbohydrate benzyl alkyl ethers do not occur; evidence of the occurrence of such direct cell wall polymer cross-linking structures has been reported (Eriksson *et al.* 1980; Fengel and Wegener 1989; Watanabe *et al.* 1989). It is conceivable that quinone methides formed during lignification of carbohydrate-rich regions of the cell wall react with e.g. hemicellulosic hydroxyls, which are not in competition with phenolic hydroxyls, to form non-cyclic benzyl alkyl ether bonds (Freudenberg 1968; Tanaka *et al.* 1979; Katayama *et al.* 1980). Etherification of preformed benzyl alcohols upon aging of lignin has also been proposed (Hemmingson 1979; Leary *et al.* 1983).

Conclusions

Both the β-5-coupled and the β-β-coupled dehydrodiconiferyl alcohols **1a** and **2** can be conveniently prepared *via* silver (I) oxide oxidation of coniferyl alcohol. Whereas the formation of **1a** predominates in methylene chloride, a good yield of **2** (pinoresinol) was obtained when the Ag_2O-mediated oxidative coupling of coniferyl alcohol was performed in 1 : 1 methylene chloride : water solution. The observation of substantial quantities of non-cyclic α-O-4 benzyl aryl ethers may have implications regarding the potential significance of these structures in "bulk" lignins, presumably found in middle lamella and cell corner regions during cell wall development.

Experimental

Melting points are uncorrected. Evaporations were conducted under reduced pressure at temperatures less than 42 °C unless otherwise noted. Further elimination of organic solvents, as well as drying of the residues, was accomplished under high vacuum (90–120 mtorr) at room temperature. TLCs were done with Alugram Sil-G/UV_{254} plates (Macherey-Nagel), with visualization by *uv* light. Column chromatography was performed on silica gel 60 (230–400 mesh, EM Science) and preparative TLC was on silica gel 60 F-254 pre-coated plates (E. Merck). Acetylations were performed using the acetic anhydride/4-(dimethylamino)pyridine procedure (Ralph *et al.* 1992b). The buffered aqueous solutions were obtained using standard citrate buffers (2.0 M citrate). The pH values were measured with a Corning model 250 ion analyser. NMR spectra of samples in acetone-d_6 (unless otherwise noted) were run at 300 K on a Bruker AMX-360 360 MHz narrow-bore instrument fitted with a 5 mm QNP probe with normal geometry. The central solvent signals were used as internal reference (^{1}H, 2.04 ppm; ^{13}C, 29.80 ppm). One- and two-dimensional NMR spectra were obtained using standard pulse sequence programs. ^{1}H-^{13}C one-bond and long-range chemical shift correlation experiments were performed in the inverse detection mode by using Bruker's invbtp and inv4lplrnd pulse programs, respectively (Bax and Subramanian 1986; Bax and Summers 1986). Assignments of the *erythro/threo* stereochemistry of the benzyl alcohols **4** was based on the chemical shifts of the non-equivalent geminal γ-protons, the *threo* γ-proton resonances being typically upfield of their *erythro* counterparts (Nakatsubo and Higuchi 1975b; Ralph and Helm 1991). This trend also applies to non-cyclic benzyl aryl ether compounds (Johansson and Miksche 1972; Brunow *et al.* 1989; Helm and Ralph 1992); the *erythro* configuration of **5–7** was thus deduced from the chemical shifts of their γ-protons (unit A), as compared to those of the benzyl alcohol (**4**) γ-protons. TOCSY spectra were run by using Bruker's mlevtp pulse program (Bax and Davis 1985) and used to assign the side-chain protons of each isomer of **4**. The chemical shifts and coupling constants of γ-proton signals of compounds **4–7** overlapping with methoxyl signals were obtained by reprocessing selected 1D rows of 2D TOCSY spectra (Helm and Ralph 1992).

Typical procedure for the silver (I) oxide oxidation of coniferyl alcohol

Silver (I) oxide (1.5 eq) was added to a solution (3–5 ml) of coniferyl alcohol (typically 100–150 mg) in the chosen solvent (Quideau and Ralph 1992). The different reaction solvents used are given in Table 1, as well as the reaction times. The pH values of aqueous solutions were measured at the beginning and at the end of the reaction, which was monitored by TLC (5 % CH_3OH–CH_2Cl_2). The reaction mixture was stirred until complete disappearance of coniferyl alcohol, and was then filtered through a bed of celite, extracted with ethyl acetate and washed with saturated aqueous NaCl, dried over $MgSO_4$ and evaporated to dryness. The residue was then submitted to preparative TLC (5 % CH_3OH–CH_2Cl_2, multiple elution) to afford, from the fastest to the slowest fraction: coniferaldehyde (traces), pinoresinol **2** [crystallization from acetone-CCl_4 gave **2** as white needles, mp 109–110 °C (lit. 118–120 °C, Pelter *et al.* 1982)], ^{1}H and ^{13}C NMR criteria agreed with published data (Lüdemann and Nimz 1974a; Vande Velde *et al.* 1984), unreacted coniferyl alcohol (traces), the aldehydic phenylcoumaran **1b** (traces), *erythro*-guaiacylglycerol-α-pinoresinol-β-coniferyl-bis-ether **7**, dehydrodiconiferyl alcohol **1a**, guaia-

Table 3. Selected ^{1}H and ^{13}C NMR data for the non-cyclic benzyl aryl ether compounds[a,b]

Carbon/proton(s)		**5** *erythro*	**6** *erythro*	**7** *erythro*
unit A:	α[c]	81.15 5.46 *d* ($J_{\alpha\beta}$ = 5.5 Hz)	81.08 5.47 d ($J_{\alpha\beta}$ = 5.7 Hz)	81.21 5.45 *d* ($J_{\alpha\beta}$ = 5.6 Hz)
	β	85.31 4.56 *brq*	85.19 4.58 *brq*	85.38 4.55 *brq*
	γ	61.75 3.81 *dd* ($J_{\gamma1\beta}$ = 7.1 Hz) 3.93 *dd* ($J_{\gamma2\beta}$ = 4.1 Hz) ($J_{\gamma1\gamma2}$ = 11.7 Hz)	61.76 3.84 *dd* ($J_{\gamma1\beta}$ = 7.1 Hz) 3.92 *dd* ($J_{\gamma2\beta}$ = 4.5 Hz) ($J_{\gamma1\gamma2}$ = 11.8 Hz)	61.82 3.82 *dd* ($J_{\gamma1\beta}$ = 6.5 Hz) 3.92 *dd* ($J_{\gamma2\beta}$ = 5.2 Hz) ($J_{\gamma1\gamma2}$ = 11.5 Hz)
unit C:	α	–	88.15 5.54 ($J_{\alpha\beta}$ = 6.3 Hz)	86.37, 86.60[d] 4.63 *brq*
	β	–	54.67 3.48 *brq*	55.15 3.04 *m*
	γ	–	64.64 3.78 *brdd* ($J_{\gamma1\beta}$ = 6.5 Hz) 3.85 *dd* ($J_{\gamma2\beta}$ = 4.7 Hz) ($J_{\gamma1\gamma2}$ = 10.5 Hz)	72.23 3.77 *m* 4.17 *m*

[a] Chemical shifts are given in ppm (acetone-d_6). [b] E.g. unit A refers to the phenylpropanoid unit with aromatic ring A; see figures for atom and aromatic ring designations. [c] The first set of values corresponds to carbon chemical shifts, the second set to proton chemical shifts. Coupling constants are given in parentheses; see Experimental for details. [d] Chemical shift of carbon-α of unit D.

cylglycerol-β-coniferyl ether **4** (*erythro* : *threo* mixture), *erythro*-guaiacylglycerol-α,β-bis-coniferyl ether **5** and *erythro*-guaiacylglycerol-α-dehydrodiconiferyl-β-coniferyl-bis-ether **6**. The benzyl aryl ether **7** was initially obtained in admixture with **1a**. Further purification was accomplished by preparative TLC eluting with $CHCL_3$–EtOAc (1 : 1 by vol.). The yield of the main reaction products are given in Table 1. Selected ^{1}H- and ^{13}C-NMR data for the benzyl aryl ethers **5–7** are given in Table 3.

Phenylcoumaran **1b:** ^{1}H NMR: δ 3.62 (1 H, brq, H-β) 3.82–3.92 (3H, m, H-γ and OH-γ) 3.82 (3H, s, MeO-A3) 3.91 (3H, s, MeO-B3) 5.65 (1 H, d, J = 6.6 Hz, H-α) 6.65 (1 H, dd, J = 15.8 and 7.7 Hz, H-β′) 6.81 (1 H, d, J = 8.1 Hz, A-5) 6.88 (1 H, ddd, J = 8.1, 2.0 and 0.5 Hz, A-6) 7.04 (1 H, d, J = 2.0 Hz, A-2) 7.29 (1 H, brs, B-2) 7.32 (1 H, brs, B-6) 7.59 (1 H, d, J = 15.8 Hz, H-α′) 7.71 (1 H, brs, A4-OH) 9.63 (1 H, d, J = 7.7 Hz, aldehydic H-γ′); ^{13}C NMR : δ 54.25 (β) 56.29 (A3-OMe) 56.46 (B3-OMe) 64.32 (γ) 89.39 (α) 110.59 (A-2) 113.56 (B-2) 115.76 (A-5) 119.64 (B-6) 119.73 (A-6) 127.14 (β′) 129.00 (B-5) 131.24 (B-1) 133.75 (A-1) 145.65 (B-3) 147.55 (A-4) 148.46 (A-3) 152.41 (B-4) 154.10 (α′) 193.77 (γ′).

Guaiacylglycerol-β-coniferyl ethers **4:** ^{1}H NMR (40 : 60 *erythro* : *threo* mixture), *threo*: δ 3.48 (1 H, dd, J = 11.9, 5.7 Hz, H-γ_1) 3.67 (1 H, dd, J = 11.9, 3.7 Hz, H-γ_2) 3.80–3.90 (2 H, OH-γ and OH-γ′) 3.80 (3 H, s, MeO-A3) 3.88 (3 H, s, MeO-B3) 4.20 (2 H, brdd, J = 5.3, 1.6 Hz, H-γ′) 4.20 (1 H, m, H-β) 4.45 (1 H, d, J = 3.9 Hz, OH-α) 4.87 (1 H, brd, J = 5.5 Hz, H-α) 6.28 (1 H, dt, J = 15.9, 5.4 Hz, H-β′) 6.52 (1 H, dt, J = 15.9, 1.6 Hz, H-α′) 6.76 (1 H, d, J = 8.1 Hz, A-5) 6.88–6.91 (2 H, m, A-6 and B-6) 7.08 (1 H, d, J = 1.9 Hz, A-2) 7.09 (1 H, d, J = 1.9 Hz, B-2) 7.11 (1 H, d, J = 8.4 Hz, B-5) 7.50 (1 H, brs, A4-OH); *erythro*: δ 3.69 (1 H, dd, J = 11.8, 4.0 Hz, H-γ_1) 3.81 (1 H, dd, J = 11.8, 6.2 Hz, H-γ_2) 3.80–3.90 (2 H, OH-γ and OH-γ′) 3.80 (3 H, s, MeO-A3) 3.84 (3 H, s, MeO-B3) 4.19 (2 H, brdd, J = 5.4, 1.6 Hz, H-γ′) 4.29 (H, m, H-β) 4.57 (1 H, d, J = 4.6 Hz, OH-α) 4.89 (1 H, brd, J = 5.2 Hz, H-α) 6.26 (1 H, dt, J = 15.9, 5.4 Hz, H-β′) 6.50 (1 H, dt, J = 15.9, 1.6 Hz, H-α′) 6.75 (1 H, d, J = 8.1 Hz, A-5) 6.86–6.89 (2 H, m, A-6 and B-6) 6.91 (1 H, d, J = 1.9 Hz, B-5) 7.05 (1 H, d, J = 1.9 Hz, B-2) 7.10 (1 H, d, J = 8.4 Hz, A-2) 7.47 (1 H, brs, A4-OH); ^{13}C NMR, *threo*: δ 56.19 (A3-OMe) 56.31 (B3-OMe) 61.88 (γ) 63.25 (γ′) 73.83 (α) 88.37 (β) 110.85 (B-2) 111.42 (A-2) 115.21 (A-5) 119.57 (B-5) 120.31 (B-6) 120.54 (A-6) 129.63 (β′) 129.85 (α′) 132.96 (B-1) 133.82 (A-1) 146.83 (A-4) 148.03 (A-3) 149.15 (B-4) 151.69 (B-3); *erythro*: δ 56.21 (A3-OMe) 56.29 (B3-OMe) 61.82 (γ) 63.25 (γ′) 73.81 (α) 86.63 (β) 110.99 (B-2) 111.44 (A-2) 115.13 (A-5) 119.27 (B-5) 120.23 (B-6) 120.46 (A-6) 129.54 (β′) 129.87 (α′) 132.84 (B-1) 134.24 (A-1) 146.65 (A-4) 147.96 (A-3) 148.55 (B-4) 151.88 (B-3); In acetone-d_6 : D_2O, (9 : 1), a solvent mixture commonly used for NMR analyses of underivatized lignins, the *threo* β-proton signal experienced a significant downfield shift and the *erythro* α-proton signal became upfield of its *threo* counterpart. ^{1}H NMR, *threo*: δ 3.48 (1 H, dd, J = 11.8, 5.5 Hz, H-γ_1) 3.70 (1 H, dd, J = 11.8, 4.2 Hz, H-γ_2) 4.30 (1 H, m, H-β) 4.88 (1 H, d, J = 5.3 Hz, H-α); *erythro*: δ 3.71 (1 H, dd, J = 12.2, 5.5 Hz, H-γ_1) 3.81 (1 H, dd, J = 12.2, 6.7 Hz, H-γ_2) 4.34 (1 H, m, H-β) 4.85 (1 H, d, J = 5.6 Hz, H-α); ^{13}C NMR, *threo*: δ 61.14 (γ) 62.86 (γ′) 72.94 (α) 86.17 (β) 128.92 (β′) 129.96; *erythro*: δ 61.30 (γ) 62.86 (γ′) 73.19 (α) 85.36 (β) 128.89 (β′) 129.97 (α′); Pure isomers were obtained upon separation by preparative TLC [$CHCl_3$: EtOAC, (50 : 1)], following acetylation. Tetra-acetate derivatives of **4**; ^{1}H NMR, *threo*: δ 1.95 (3 H, s, OAc-γ) 2.01 (3 H, s, OAc-γ′) 2.02 (3 H, s, OAc-α) 2.21 (3 H, s, OAc-A4) 3.81 (3 H, s, MeO-A3) 3.85 (3 H, s, MeO-B3) 4.02 (1 H, dd, J = 11.9, 5.7 Hz, H-γ_1) 4.26 (1 H, dd, J = 11.9, 4.1 Hz, H-γ_2) 4.66 (2 H, dd, J = 6.4, 1.4 Hz, H-γ′) 4.81 (1 H, m, H-β) 6.10 (1 H, d, J = 6.5 Hz, H-α) 6.27 (1 H, dt, J = 15.9, 6.4 Hz, H-β′) 6.63 (1 H, dt, J = 15.9, 1.4 Hz, H-α′) 6.95 (1 H, dd, J = 8.3, 2.0 Hz, B-6) 7.00 (1 H, d, J = 8.3 Hz, B-5) 7.03 (1 H, d, J = 8.1 Hz, A-5) 7.06 (1 H, dd, J = 8.1, 1.8 Hz, A-6) 7.14 (1 H, d, J = 2.0 Hz, B-2) 7.22 (1 H, d, J = 1.8 Hz, A-2); *erythro*: δ 1.93 (3 H, s, OAc-γ) 2.02 (3 H, s, OAc-γ′) 2.07 (3 H, s, OAc-α) 2.21 (3 H, s, OAc-A4) 3.82 (3 H, s, MeO-A3) 3.85 (3 H, s, MeO-B3) 4.22 (1 H, dd, J = 11.9, 4.1 Hz, H-γ_1) 4.36 (1 H, dd, J = 11.9, 5.9 Hz, H-γ_2) 4.66 (2 H, dd, J = 6.4, 1.4 Hz, H-γ′) 4.85 (1 H, m, H-β) 6.06 (1 H, d, J = 5.1 Hz, H-α) 6.26 (1 H, dt, J = 15.9, 6.4 Hz, H-β′) 6.62 (1 H, dt, J = 15.9, 1.4 Hz, H-α′) 6.93 (1 H, dd, J = 8.3, 2.0 Hz, B-6) 6.97 (1 H, d, J = 8.3 Hz, B-5) 7.02 (1 H, d, J = 8.1 Hz, A-5) 7.05 (1 H, dd, J = 8.1, 1.8 Hz, A-6) 7.13 (1 H, d, J = 2.0 Hz, B-2) 7.25 (1 H, d, J = 1.8 Hz, A-2); ^{13}C NMR, *threo*: δ 20.44 (OCO*Me*-A4) 20.58 (OCO*Me*-γ) 20.78 (OCO*Me*-α) 20.91 (OCO*Me*-γ′) 56.24 (A3-

OMe) 56.29 (B3-OMe) 63.56 (γ) 65.36 (γ′) 75.32 (α) 80.63 (β) 111.31 (B-2) 112.64 (A-2) 118.74 (B-5) 120.30 (A-6) 120.54 (B-6) 123.26 (β′) 123.56 (A-5) 132.35 (B-1) 134.16 (α′) 136.61 (A-1) 140.92 (A-4) 149.09 (B-4) 151.75 (B-3) 152.22 (A-3) 168.89 (OCOMe-A4) 170.00 (OCOMe-α) 170.68 (OCOMe-γ) 170.77 (OCOMe-γ′); *erythro*: δ 20.45 (OCO*Me*-A4) 20.59 (OCO*Me*-γ) 20.77 (OCO*Me*-α) 20.85 (OCO*Me*-γ′) 56.25 (A3-OMe) 56.27 (B3-OMe) 62.98 (γ) 65.34 (γ′) 74.49 (α) 80.27 (β) 111.38 (B-2) 112.73 (A-2) 119.36 (B-5) 120.39 (A-6) 120.48 (B-6) 123.35 (A-5) 123.37 (β′) 132.63 (B-1) 134.12 (α′) 136.59 (A-1) 140.79 (A-4) 148.27 (B-4) 151.98 (B-3) 152.11 (A-3) 168.90 (OCOMe-A4) 169.88 (OCOMe-α) 170.74 (OCOMe-γ′ and OCOMe-γ).

Synthesis of dehydrodiconiferyl alcohol **1a**

Silver (I) oxide (290 mg, 1.25 mmol) was added to a solution of coniferyl alcohol (150 mg, 0.83 mmol) in CH_2Cl_2 (5 ml, dried by passage through alumina). After being stirred for 24 h (monitoring by TLC, 5% CH_3OH–CH_2Cl_2), the reaction mixture was filtered though a bed of Celite and evaporated to dryness to give a yellow foam in quantitative yield. This residue was submitted to flash chromatography [$CHCl_3$: EtOAc (1 : 1)] to afford, after crystallization from acetone-light petroleum (boiling range 40–60 °C), **1a** (74 mg, 50%) as white needles; mp 164–165 °C (lit. 156–157 °C, Freudenberg and Schlüter 1955); ^{1}H NMR: δ 3.53 (1 H, brq, H-β) 3.78–3.88 (3 H, m, H-γ and OH-γ) 3.81 (3 H, s, MeO-A3) 3.85 (3 H, s, MeO-B3) 4.16 (1 H, t, J = 5.0 Hz, OH-γ′) 4.19 (2 H, td, J = 5.2 and 1.5 Hz, H-γ′) 5.56 (1 H, d, J = 6.5 Hz, H-α) 6.23 (1 H, dt, J = 15.8 and 5.5 Hz, H-β′) 6.52 (1 H, dt, J = 15.8 and 1.5 Hz, H-α′) 6.80 (1 H, d, J = 8.1 Hz, A-5) 6.87 (1 H, ddd, J = 8.1, 2.0 and 0.5 Hz, A-6) 6.94 (1 H, brs, B-2) 6.97 (1 H, brs, B-6) 7.03 (1 H, d, J = 2.0 Hz, A-2) 7.73 (1 H, brs, A4-OH); ^{13}C NMR: δ 54.73 (β) 56.26 (A3-OMe) 56.38 (B3-OMe) 63.39 (γ′) 64.60 (γ) 88.51 (α) 110.48 (A-2) 111.72 (B-2) 115.67 (A-5) 116.08 (B-6) 119.57 (A-6) 128.33 (β′) 130.40 (B-5) 130.54 (α′) 131.91 (B-1) 134.36 (A-1) 145.14 (B-3) 147.27 (A-4) 148.36 (A-3) 148.94 (B-4); [acetone-d_6 : D_2O, (9 : 1), selected data] ^{1}H NMR: δ 3.48 (1 H, brq, H-β) 4.14 (2 H, dd, J = 5.7 and 1.4 Hz, H-γ′) 5.53 (1 H, d, J = 6.2 Hz, H-α) 6.20 (1 H, dt, J = 15.8 and 5.7 Hz, H-β′) 6.52 (1 H, dt, J = 15.8 and 1.4 Hz, H-α′); ^{13}C NMR: δ 54.39 (C-β) 62.97 (C-γ′) 64.25 (C-γ) 88.32 (C-α) 127.86 (β′) 130.61 (α′). Tri-acetate derivative of **1a**; ^{1}H NMR: δ 2.0 (3 H, s, OAc-γ) 2.01 (3 H, s, OAc-γ′) 2.22 (3 H, s, OAc-A4) 3.78 (1 H, m, H-β)) 3.79 (3 H, s, MeO-A3) 3.88 (3 H, s, MeO-B3) 4.34 (1 H, dd, J = 11.1 and 7.6 Hz, H-γ_1) 4.46 (1 H, dd, J = 11.1 and 5.4 Hz, H-γ_2) 4.66 (2 H, dd, J = 6.5 and 1.3 Hz, H-γ′) 5.61 (1 H, d, J = 6.7 Hz, H-α) 6.24 (1 H, dt, J = 15.8 and 6.5 Hz, H-β′) 6.63 (1 H, dt, J = 15.8 and 1.3 Hz, H-α′) 7.0 (1 H, brdd, J = 8.1 and 1.8 Hz, A-6) 7.03 (1 H, brs, B-6) 7.049 (1 H, brs, B-2) 7.054 (1 H, d, J = 8.1 Hz, A-5) 7.18 (1 H, d, J = 1.8 Hz, A-2; ^{13}C NMR: δ 20.43 (OCO*Me*-A4) 20.67 (OCO*Me*-γ) 20.78 (OCO*Me*-γ′) 51.33 (β) 56.24 (A3-OMe) 56.41 (B3-OMe) 65.47 (γ′) 65.96 (γ) 88.34 (α) 111.07 (A-2) 112.20 (B-2) 116.32 (B-6) 118.67 (A-6) 122.30 (β′) 123.76 (A-5) 128.82 (B-5) 131.65 (B-1) 134.64 (α′) 140.70 (A-4) 140.99 (A-1) 145.39 (B-3) 149.21 (B-4) 152.38 (A-3) 168.98 (OCOMe-A4) 170.80 (OCOMe-γ′) 170.97 (OCOMe-γ).

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