Oxidative cross coupling of *p*-hydroxycinnamic alcohols with dimeric arylglycerol β-aryl ether lignin model compounds. The effect of oxidation potentials

Kaisa Syrjänen and Gösta Brunow *

Laboratory of Organic Chemistry, Department of Chemistry, PO Box 55, FIN-00014 University of Helsinki, Finland

Received (in Cambridge) 9th July 1998, Accepted 25th August 1998

Oxidation of *p*-hydroxycinnamyl alcohols together with dimeric lignin model compounds containing arylglycerol β-aryl ether structures was found, under certain conditions, to yield cross coupling products with β-aryl ether and 5,5′-biphenyl bonds. Cross coupling appeared to be restricted to phenols of similar oxidation potential. The implications of the "limits to randomness" for the understanding of the lignification process in vascular plants are discussed.

Introduction

The final step in the formation of lignin in cell walls of vascular plants is a dehydrogenative polymerization of substituted *p*-hydroxycinnamyl alcohols. The reaction is initiated by an oxidative enzyme (peroxidase or laccase), but the polymerization itself is currently viewed as being a process without enzymatic control over the distribution of structural units. The intractable nature of the cell wall structure in lignified plants makes it difficult to prepare samples of unchanged lignin for structural studies. For this reason, the study of the lignification reaction constitutes an important key to the understanding of the structure of lignin.[1,2] The unraveling of the order of events during lignin deposition in cell wall biosynthesis will yield important information on the distribution of structural units, branching points and end groups.

During lignification, the bond formation process is an oxidative phenol coupling. The oxidative dimerisation of *p*-hydroxycinnamyl alcohols is well documented; the C–O and C–C bonds that are formed reflect the reactivities of the phenoxyl radicals that are formed on oxidation. The main products are termed β-5, β-O-4 and β-β dimers (illustrated for coniferyl alcohol in the upper part of Scheme 1, structures **3**, **4** and **9**, respectively). The further growth to oligomer and polymer stages is less well understood. This process is no longer a coupling of two identical radicals, it is a reaction between two different phenols: a cross coupling (see Scheme 2).

To study this process, we have prepared three lignin precursors, coniferyl, sinapyl and *p*-coumaryl alcohol (**1a**–**1c**), and three β-O-4 dimers (**2a**–**2c**), to represent phenol end groups on the growing polymer with zero, one and two *o*-methoxy groups, respectively. We oxidized equimolar mixtures of the *p*-hydroxycinnamyl alcohols and dimeric models under conditions assumed to prevail in lignin biosynthesis. Cross coupling proved surprisingly difficult to achieve. Cross coupled products were isolated only in two cases, with the formation of β-O-4 and 5,5′-biphenyl structures. The best yields of cross coupling products were obtained when coniferyl alcohol was coupled with a syringyl model dimer. We found that the results can be rationalized by assuming that cross coupling is governed by a combination of factors such as oxidation potential and radical reactivity.

Results

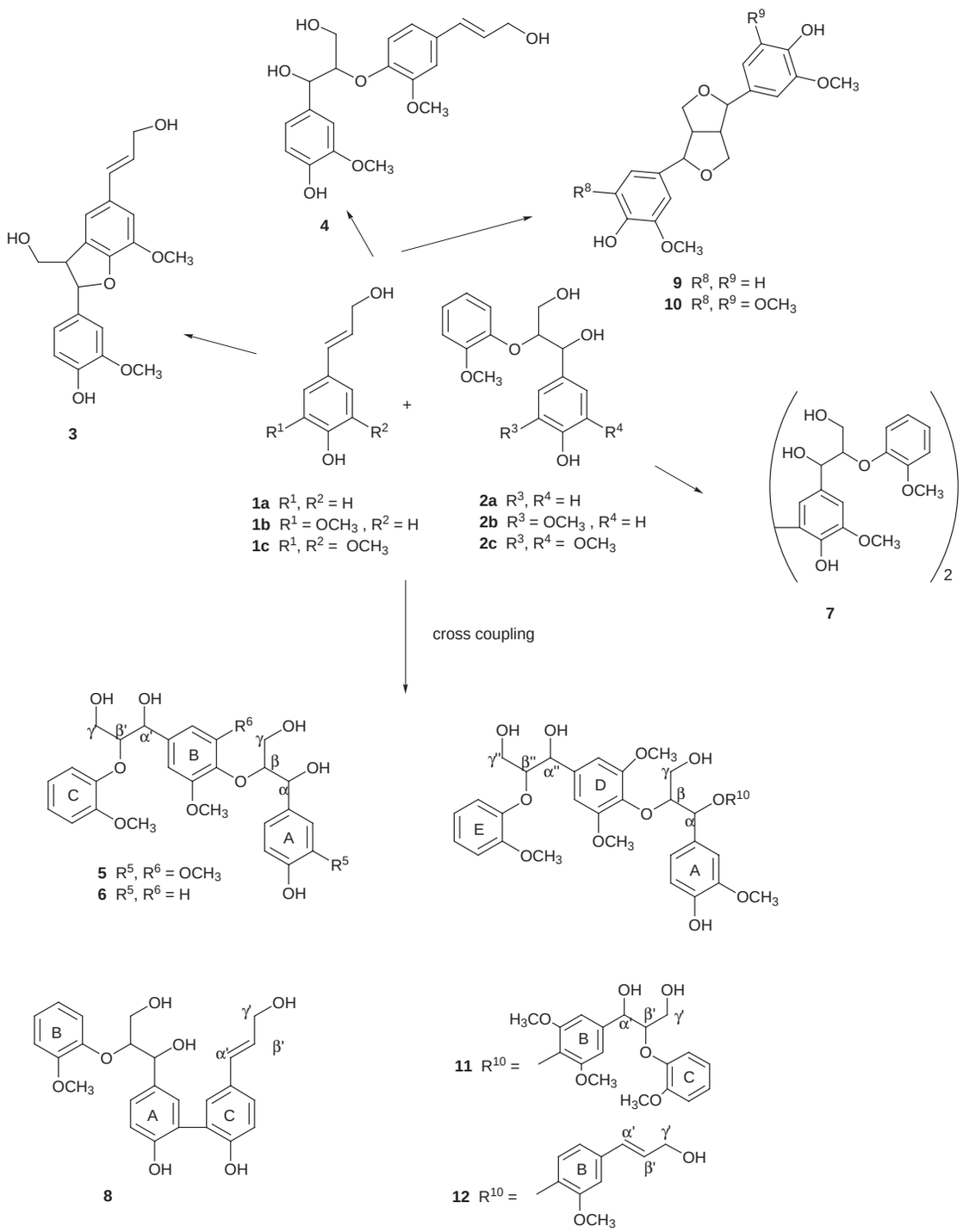
The oxidations were carried out with hydrogen peroxide, catalyzed by horseradish peroxidase, in solutions buffered to pH 3.5. The pH was kept lower than the optimum for horseradish peroxidase because we have previously[3] observed that lower pH favours the formation of dimers at the expense of polymeric products. There is also experimental evidence that suggests that the pH in tissues undergoing lignification is lower than 4.[4] The *p*-hydroxycinnamyl alcohol (**1a**), coniferyl alcohol (**1b**) and sinapyl alcohol (**1c**) were each oxidized in the presence of an equimolar amount of one of the dimers **2a**–**2c**. Some organic solvent (dioxane or acetone) was added to dissolve the starting materials. The amount of organic solvent was kept as low as possible to prevent denaturation of the enzyme. The products were acetylated and isolated using preparative HPLC and identified with the aid of NMR and mass spectral analysis. Additional experiments were carried out with the enzyme in wet chloroform as solvent and finally with silver oxide as oxidant. In all experiments, variable amounts of oligomeric products were obtained that were not characterized further. The results are summarized in Table 1.

Oxidation with coniferyl alcohol

The oxidation of a mixture of coniferyl alcohol and the *p*-coumaryl dimer **2a** resulted in no cross coupling. The coupling products were from coniferyl alcohol alone: 10% of β-5 product **3** was isolated, while the β-O-4 and β-β dimers **4** and **9** (10%) were eluted together. Dimer **2a** was to a large extent recovered unchanged. With the guaiacyl dimer **2b** the result was similar: 20% of the β-O-4 dimer **4** and some 5-5 dimerization product (**7**)[5] from **2b** were isolated, together with large amounts of unchanged dimer **2b** and some **3**. No cross coupling products were found. With the syringyl dimer **2c**, the cross coupling was the main reaction. The product, which was obtained in 40% yield, was assigned the β-O-4 coupled trimer structure **5** on the basis of NMR and mass spectral data. Compound **5** was obtained as a mixture of diastereomers. The starting material **2c** was mainly the *erythro* isomer and that stereostructure was retained in the trimer **5**. The new β-O-4 structure in **5** proved to be a mixture of almost equal amounts of *erythro* and *threo*.

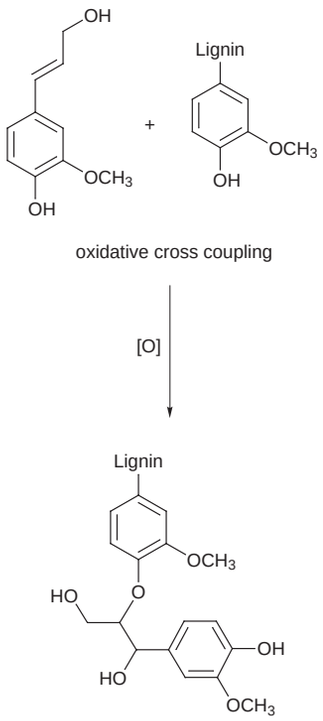
Table 1 Oxidative coupling of *p*-hydroxycinnamyl alcohols **1a**–**1c** with β-O-4 dimers **2a**–**2c**. For reaction conditions, see text

Experiment	Solvent	Starting phenols	Products (yield %)
1	water	**1b**, **2a**	**2a** (50%), **3** (10%), **4** + **9** (10%)
2	water	**1b**, **2b**	**2b** + **3** (45%), **4** (20%), **7** (10%)
3	water	**1b**, **2c**	**2c** (25%), **3** (10%), **4** (20%), **5** (40%)
4	water	**1c**, **2c**	**2c** (50%), **10** (25%)
5	water	**1c**, **2b**	**2b** (50%), **10** (10%)
6	water	**1a**, **2b**	**2b** (30%), **8** (7%), **6** (10%), **7** (7%)
7	$CHCl_3$	**1b**, **2c**	**11** (14%)
8	CH_2Cl_2	**1b**, **2c**	**11** (15%), **12** (14%)



Scheme 1 Structures of products from oxidative coupling of *p*-hydroxycinnamyl alcohols **1a**–**1c** with β-O-4 dimers **2a**–**2c**.

This is in contrast to the predominance of *erythro* structures that has been observed in hardwood lignins.[6] Dimers of coniferyl alcohol were also isolated together with some unchanged **2c**. Oxidation of mixtures of **1b** and **2c** in organic solvents (experiments 7 and 8, Table 1) yielded pentameric and tetrameric compounds **11** and **12** that contained α-O-4 structures.



Scheme 2

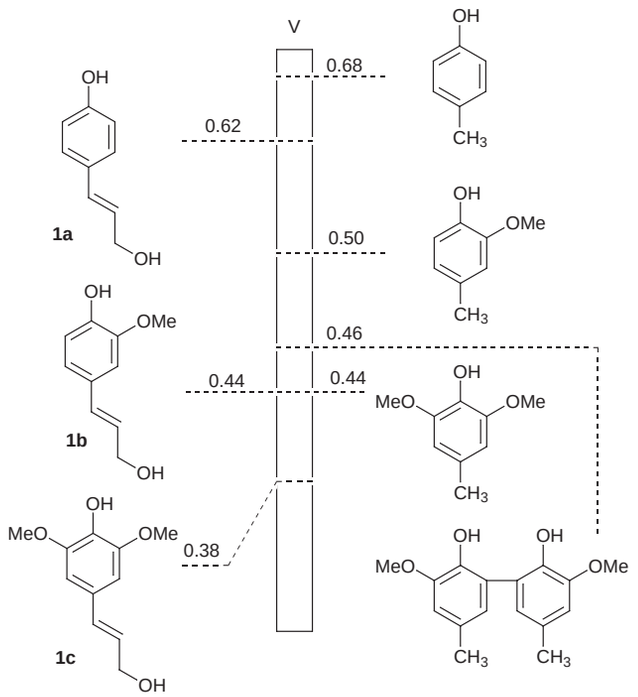


Fig. 1 Estimated oxidation potentials of methoxy and propenyl substituted phenols. For explanation, see text.

These are probably formed because the addition of water to the intermediate quinone methide[7] is suppressed under the oxidation conditions.

Oxidation with sinapyl alcohol

No cross coupling products were obtained in oxidations with dimers **2b** or **2c**. The main coupling product was syringaresinol. Some oligomeric products were obtained that were not further characterized.

Oxidation with *p*-coumaryl alcohol

This alcohol was oxidized together with the guaiacyl dimer **2b**. Unchanged **2b** was the main product in this case together with some of its 5-5 dimer **7** (7%). Two cross coupled products were isolated: the β-O-4 coupled trimer **6** (10%) and the 5-5 coupled trimer **8** (7%). The formation of **8** is of interest because the formation of 5-5 dimers from propenylphenols has not been previously observed.[8]

Discussion

The results demonstrate that cross coupling between lignin precursors and the lignin polymer occurs only under very restricted circumstances, and cannot be regarded as a random process. One important restriction is the oxidation potentials of the phenols (or phenolates) involved. Fig. 1 shows an estimate of the influence of some substituents on the oxidation potentials of the phenols. It has been constructed by extrapolation of data from two references.[9,10] The numbers on the right are approximations of substituent effects taken from ref. 9; the numbers on the left are taken from ref. 10 and refer to the oxidation potential of the phenolate. When a mixture of phenols is oxidized, the phenols will react in the order shown, starting with the lowest on the scale. Significant cross coupling is expected with phenols on the same level on the potential scale, in other cases they will react one after the other, without any cross coupling. Our experiments show that radicals from *p*-hydroxycinnamyl alcohols such as the lignin precursors **1a**–**1c** dimerize very rapidly and that the coupling invariably involves the β-carbon in the side chain, which probably has a high reactivity. In equimolar mixtures with other phenols, cross coupling only occurs within a restricted range of oxidation potential. The successful coupling of **1b** and **2c** can be understood by comparing the oxidation potentials in Fig. 1; the same reasoning explains the cross coupling between **1b** and 5,5′-biphenyl dimers, where dibenzodioxocins are formed in good yields.[11] In the reaction of *p*-hydroxycinnamic alcohol **1a** with dimer **2b**, some cross coupling was observed in spite of a large difference in oxidation potential. In this case, the phenol with the higher oxidation potential has a more reactive side chain. This may be an indication that other effects, such as radical reactivity, also may be important factors.

It is still unclear under what conditions the couplings occur that are not favoured by similarity of oxidation potentials. The preponderance of β-O-4 structures in guaiacyl lignins shows that during biosynthesis, coupling occurs between coniferyl alcohol and guaiacyl groups in the polymer. This we were unable to achieve in our present experiments. It is presently assumed[1] that cross coupling can be achieved by manipulating the concentrations of the component phenols. Dehydrogenated polymers of coniferyl alcohol with high amounts of β-O-4 structures have been obtained by keeping the concentration of the cinnamyl alcohol component low during the reaction ("Zutropf"), but exact reproduction of natural lignins has not been achieved.[12] It seems to be difficult to reproduce the conditions of "infinite dilution" of the precursors that probably prevail in the lignifying cell wall. We believe that the study of the conditions of radical cross coupling will yield important information on the distribution of structural elements and the "limits to randomness" in the organization of cell wall lignin.

Experimental

Melting points, determined in open-capillary tubes with an electrothermal apparatus, are uncorrected. Horseradish peroxidase (E 1.11.1.7) was from Serva, activity 250 or 450 U mg^{-1}. 30% Aqueous hydrogen peroxide (Merck) was diluted to

give a 3% solution (*ca.* 0.8 mol cm^{-3}) before use. Silica gel for column chromatography was Merck Kieselgel 60 (230–400 mesh). Thin layer chromatography was performed on silica gel plates (Merck Kieselgel 60 F_{254}). Spots were made visible with UV light. Preparative HPLC was performed using an ISCO model 2350 HPLC pump, a LiChrospher Si 60 (5 mm) column (1 × 25 cm) and a Shimadzu UV spectrophotometric SPD-6A detector with detection at 260 nm. Hexane–ethyl acetate was used as eluent. The injection volume was 1 cm^3. ^1H NMR and ^{13}C NMR spectra were recorded at 200 MHz with a Varian Gemini instrument. Deuteriochloroform was used as solvent and tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a JEOL JMS-SX102 instrument. Elemental analyses were performed by Analytische Laboratorien Malissa und Reuter, Lindlar, Germany. Evaporations were conducted under reduced pressure at temperatures below 40 °C. Products were acetylated with dry acetic anhydride and pyridine (1 : 1) overnight at room temperature.[13]

Preparation of starting materials

4-(3-Hydroxyprop-1-enyl)phenol **1a** (*p*-coumaryl alcohol), 4-(3-hydroxyprop-1-enyl)-2-methoxyphenol **1b** (coniferyl alcohol) and 4-(3-hydroxyprop-1-enyl)-2,6-dimethoxyphenol **1c** (sinapyl alcohol) were prepared by reduction of the corresponding cinnamic esters.[14,15]

1-(4-Hydroxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol **2a**, 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol **2b** and 1-(3,5-dimethoxy-4-hydroxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol **2c** were prepared using syntheses based on the method of Nakatsubo *et al.*[16,17] and have been published earlier.[18]

Oxidations with horseradish peroxidase/H_2O_2

General procedure. Monomer (**1a**–**1c**) (2.0 mmol) and dimer (**2a**–**2c**) (2.0 mmol) were dissolved in acetone (30 cm^3) and horseradish peroxidase (10 mg; 450 U mg^{-1}) in buffer solution (120 cm^3; pH 3.5) was added under argon atmosphere. H_2O_2 (1.0 mmol) diluted to 10 cm^3 with buffer solution was added over 30 min at room temperature, and then the mixture was stirred for an additional 30 min. The mixture was extracted with methylene chloride, washed with water, dried with Na_2SO_4 and the solvent was evaporated. The residue was acetylated. Products were separated and purified using preparative HPLC (eluent: AcOEt–hexane, 3 : 2, phenols **1a** and **2b** AcOEt–hexane, 4 : 3) and analyzed by NMR and MS.

Peracetate of trimer 5. A colourless oil; δ_H(200 MHz) 1.94–2.13 (12H, m, -$COCH_3$), 2.29 (3H, s, -$COCH_3$), 3.72–3.82 (9H, m, -OCH_3), 4.16–4.71 (6H, m, β-CH, β′-CH, γ-CH_2, γ′-CH_2), 5.98 (1H, d, α′-CH), 6.04–6.16 (1H, m, α-CH), 6.57–7.08 (9H, m, arom.); δ_C(50 MHz) 20.7, 20.8, 21.0 (-$COCH_3$), 55.8, 55.9, 56.0 (-OCH_3), 62.7, 62.8, 63.49, 63.54 (γ-CH_2, γ′-CH_2), 74.0, 74.2, 75.3 (α-CH, α′-CH), 80.1, 80.2, 80.7, 80.8 (β-CH, β′-CH), 104.3–153.1 (arom.), 168.9–170.9 (-$COCH_3$); *m/z* (%) 179(77), 209(52), 221(88), 222(83), 251(18), 281(27), 313(38), 323(100), 358(12), 374(11), 434(10), 636(7), 696(6), 756 (M^+, 14) (HRMS: $C_{38}H_{44}O_{16}$ requires M, 756.2629. Found: M^+, 756.2640).

Peracetate of trimer 6. An oil; δ_H(200 MHz) 1.90–2.13 (12H, m, -$COCH_3$), 2.29 (3H, -$COCH_3$), 3.71–3.86 (6H, m, -OCH_3), 4.05–4.78 (6H, m, β-CH, β′-CH, γ-CH_2, γ′-CH_2), 5.98–6.15 (2H, m, α′-CH, α-CH), 6.75–7.15 (11H, m, arom.); δ_C(50 MHz) 20.7, 20.8, 21.0, 21.1 (-$COCH_3$), 55.7, 55.8, 55.9, 56.1 (-OCH_3), 62.5, 62.7, 63.0, 63.3 (γ-CH_2, γ′-CH_2), 73.7, 74.0, 74.3, 74.7 (α-CH, α′-CH), 80.1, 80.2, 80.4 (β-CH, β′-CH), 112.4–151.0 (arom.), 169.2–170.8 (-$COCH_3$); *m/z* (%) 209(72), 293(42), 325(38), 355(38), 385(25), 415(33), 457(18), 487(7), 517(4), 576(3), 640(22), 682(37), 696 (M^+, 83) (HRMS: $C_{36}H_{40}O_{14}$ requires M, 696.2419. Found: M^+, 696.2440).

Peracetate of tetramer 7. An oil; δ_H(200 MHz): 2.00–2.29 (18H, m, -$COCH_3$), 3.74–3.83 (12H, m, -OCH_3), 4.15–4.55 (4H, m, Hγ), 4.59–4.70 (2H, m, H_β), 6.06–6.12 (2H, m, H_α), 6.78–7.06 (12H, m, arom.); ^{13}C data have been published recently;[12] *m/z* (%) 124(88), 209(60), 241(13), 329(8), 397(8), 435(10), 485(7), 503(14), 549(15), 623(6), 665(5),728(3), 767(4), 848(17), 890 (M^+, 24).

Peracetate of trimer 8. An oil; δ_H(200 MHz) 2.00–2.20 (15H, m, -$COCH_3$), 3.72–3.88 (6H, m, -OCH_3), 3.61–3.91 and 4.22–4.70 (3H, m, β-CH, γ-CH_2), 4.68–4.72 (2H, d, γ′-CH_2), 6.06–6.30 (2H, m, β′-CH, α-CH), 6.58–6.66 (1H, d, α′-CH), 6.78–7.42 (9H, m, arom.); δ_C(50 MHz) 20.3, 20.8, 21.0, 21.1 (-$COCH_3$), 55.5, 55.7, 55.9, 56.1 (-OCH_3), 62.6 (γ-CH_2), 64.9 (γ′-CH_2), 73.7 (α-CH), 80.2 (β-CH), 123.7 (β′-CH), 134.9 (α′-CH), 111.5–151.2 (arom.), 168.4, 169.4, 169.6, 170.8 (-$COCH_3$); *m/z* (%) 209(100), 283(50), 309(32), 351(52), 385(20), 411(44), 453(35), 474(15), 534(8), 555(17), 576(8), 636(49), 678 (M^+, 57) (HRMS: $C_{36}H_{38}O_{13}$ requires M, 678.2313. Found: M^+, 678.2302).

Oxidation in wet chloroform

Dimer **2c** (0.96 g, 2.7 mmol) was dissolved in wet chloroform (100 cm^3). Horse radish peroxidase (10 mg, 250 U mg^{-1}) in water (1 cm^3) was added under nitrogen. Then coniferyl alcohol **1b** (0.36 g, 2.0 mmol) in chloroform (1.3 cm^3) and H_2O_2 (2.0 mmol) diluted with water (1.3 cm^3) were added in portions at the same time to the reaction mixture. The reaction was followed by TLC, and coniferyl alcohol was added as soon as the previous batch had reacted. More enzyme had to be added when the reaction stopped (3 × 10 mg). The chloroform phase was washed with water, dried and the solvent was evaporated. The product was acetylated and then purified with column chromatography using ethyl acetate–hexane (3 : 1) as eluent to give the peracetate of **11** (160 mg, 14%), a white solid melting at 71–75 °C (Found: C, 61.09; H, 5.95. Calculated for $C_{58}H_{66}O_{23}$: C, 61.59; H, 5.88%); δ_H(200 MHz) 1.81–2.27 (18H, m, -$COCH_3$), 3.58–3.81 (21H, m, -OCH_3), 4.15–4.78 (9H, m, β-CH, β′-CH, β″-CH, γ-CH_2, γ′-CH_2, γ″-CH_2), 5.50–5.64 (1H, m, α-CH), 6.48–6.56 (2H, m, α′-CH, α″-CH), 6.48–7.03 (15H, m, arom.); δ_C(50 MHz) 20.7, 20.8, 21.0 (-$COCH_3$), 55.7, 55.8, 55.9 (-OCH_3), 62.7, 64.3, 64.5 (γ-CH_2, γ′-CH_2, γ″-CH_2), 74.0, 74.1 (α′-CH, α″-CH), 80.0, 80.1, (β-CH, β′-CH, β″-CH), 81.8 (α-CH), 104.3–152.9 (arom.), 168.8–170.9 (-$COCH_3$); *m/z* (%) 517(97), 577(90), 637(68), 697(90), 876(5), 1130 (M^+, 5).

A further fraction (30%) was unchanged starting material and the rest was oligomeric products.

Oxidation with silver(I) oxide

Silver(I) oxide (450 mg, 195 mmol) was added (argon atmosphere) to a solution of compound **1b** (230 mg, 1.3 mmol) and compound **2c** (450 mg, 1.3 mmol) in methylene chloride (26 cm^3). The reaction mixture was stirred for 30 min at room temperature, 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 acetylated, and then filtered though a bed of silica gel. Isolation of the products by preparative HPLC (eluent: AcOEt–hexane 3 : 1) yielded the peracetate of pentamer **11**, 110 mg (15%) and the peracetate of tetramer **12**, 100 mg (14%) as an oil. Spectral data for peracetate of **12**: δ_H(200 MHz) 1.89–2.10 (12H, m, -$COCH_3$), 2.29 (3H, s, -Ar$OCOCH_3$), 3.58–3.87 (15H, m, -OCH_3), 4.15–4.70 (8H, m, β-CH, β″-CH, γ-CH_2, γ′-CH_2, γ″-CH_2), 5.52 (1H, d, α-CH), 5.98 (1H, d, α″-CH), 6.05–6.20 (1H, m, β′-CH), 6.51–7.05 (13H, m, α′-CH, arom.); δ_C(50 MHz) 20.7, 20.8, 21.0 (-$COCH_3$), 55.7, 55.9 (-OCH_3), 62.8, 63.1 (γ-CH_2, γ″-CH_2), 65.2 (γ′-CH_2), 74.0 (α″-CH), 79.6, 80.1 (β-CH, β″-CH), 82.4 (α-CH), 123.5 (β′-CH), 134.2 (α′-CH), 104.4–153.1 (arom.), 168.9–170.9 (-$COCH_3$); *m/z* (%)

222(100), 252(22), 309(18), 425(4), 433(21), 485(4), 577(2), 637(29), 697(2), 858(1), 918 (M^+, 1). We were unable to obtain a high resolution mass spectrum of this compound.

References

1 K. V. Sarkanen and C. H. Ludwig, *Lignins*, *Occurrence*, *Formation*, *Structure and Reactions*, Wiley-Interscience, New York, 1971.
2 M. Nose, M. A. Bernards, M. Furlan, J. Zaijcek, T. L. Eberhardt and N. G. Lewis, *Phytochemistry*, 1995, **39**, 71.
3 F. Chioccara, S. Poli, B. Rindone, T. Pilati, G. Brunow, P. Pietikäinen and H. Setälä, *Acta Chem. Scand.*, 1993, **47**, 610.
4 J. Sipilä and G. Brunow, *Holzforschung*, 1991, **45 suppl.**, 3.
5 J. C. Pew and W. J. Connors, *J. Org. Chem.*, 1969, **34**, 580.
6 G. Brunow, O. Karlsson, K. Lundquist and J. Sipilä, *Wood Sci. Technol.*, 1993, **27**, 281.
7 J. Sipilä and G. Brunow, *Holzforschung*, 1991, **45 suppl.**, 9.
8 K. V. Sarkanen and A. F. A. Wallis, *J. Chem. Soc.*, *Perkin Trans. 1*, 1973, 1869.
9 K. Kratzl, P. Claus, W. Lonsky and J. S. Gratzl, *Wood Sci. Technol.*, 1974, **8**, 35.
10 M. Jonsson, J. Lind, T. Reitberger, T. E. Eriksen and G. Merényi, *J. Phys. Chem.*, 1993, **97**, 8229.
11 P. Karhunen, P. Rummakko, J. Sipilä, G. Brunow and I. Kilpeläinen, *Tetrahedron Lett.*, 1995, **36**, 4501.
12 L. L. Landucci, S. A. Ralph and K. E. Hammel, *Holzforschung*, 1998, **52**, 160.
13 E. Adler, G. Brunow and K. Lundquist, *Holzforschung*, 1987, **41**, 199.
14 K. Freudenberg and H. H. Hübner, *Chem. Ber.*, 1952, **85**, 1181.
15 S. Quideau and J. Ralph, *J. Agric. Food Chem.*, 1992, **40**, 1108.
16 F. Nakatsubo, K. Sato and T. Higuchi, *Holzforschung*, 1975, **29**, 165.
17 T. Katayama, F. Nakatsubo and T. Higuchi, *Mokuzai Gakkaishi*, 1981, **27**, 223.
18 J. Sipilä and K. Syrjänen, *Holzforschung*, 1995, **49**, 325.

Paper 8/05349I