A novel type of spiro compound formed by oxidative cross coupling of methyl sinapate with a syringyl lignin model compound. A model system for the β-1 pathway in lignin biosynthesis

Harri Setälä, Aarne Pajunen, Petteri Rummakko, Jussi Sipilä and Gösta Brunow *

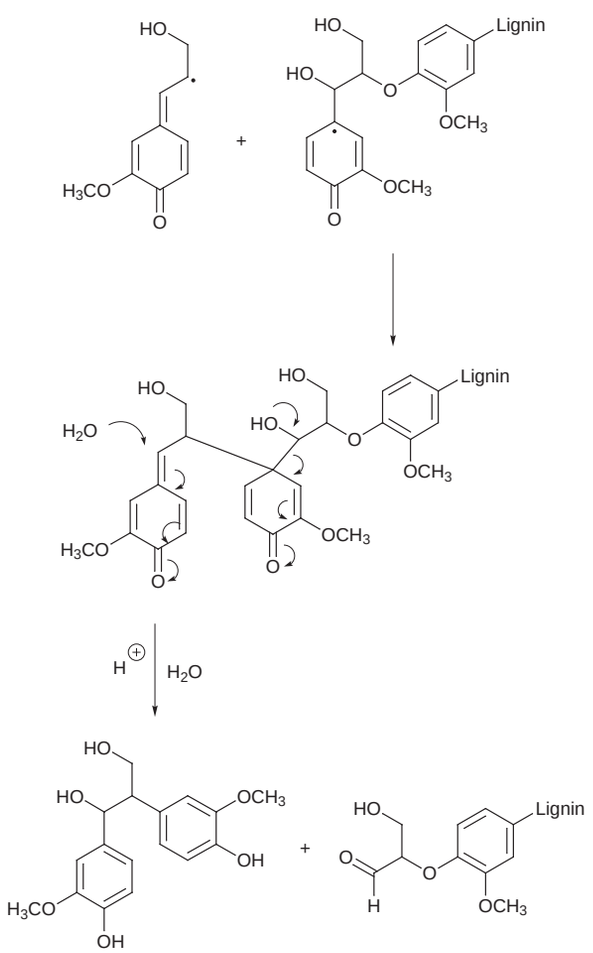
Laboratory of Organic Chemistry, Department of Chemistry, PO Box 55, FIN-00014 University of Helsinki, Finland

Received (in Cambridge) 13th November 1998, Accepted 21st December 1998

Oxidative coupling of methyl (*E*)-sinapate with 1-(4-hydroxy-3,5-dimethoxyphenyl)ethanol, a syringyl lignin model compound, was carried out with hydrogen peroxide catalyzed by horseradish peroxidase. The main product was a cyclohexadienone spiro compound of a novel type. Mild acidolysis caused the loss of one side chain and yielded a dimeric compound with a 1,2-diarylpropane structure. This is the first example of the formation of this structural type by oxidative coupling *in vitro*.

Introduction

The final step in the biosynthesis of lignin is an oxidative polymerization of phenolic precursors. In a previous article[1] we have discussed the importance of oxidation potentials and of the cross coupling of different phenolic precursors in the biosynthesis of lignin in the cell walls of woody plants. The importance of cross coupling first became apparent through the discovery of the 1,2-diarylpropane or β-1 structure in both softwood[2] and hardwood lignins.[3] These structures could not be fitted into the original dehydrogenation scheme based on the work of Erdtman and Freudenberg (for a review see ref. 4). The most plausible mechanism for the formation of these structures involves a cross coupling between a *p*-hydroxycinnamyl alcohol radical and a radical (with a saturated side chain) residing on the growing polymer chain. The intermediate cyclohexadienone was assumed to lose a side chain to form a β-1 or 1,2-diarylpropane-1,3-diol structure and a glyceraldehyde-2-aryl ether by a mechanism outlined in Scheme 1. This involves a reverse vinylogous aldol reaction and addition of water to the quinomethane group.[2] The formation of the glyceraldehyde ether stops the growth of the polymer chain. The released β-1 dimer contains phenolic groups from which a new chain may begin. Both the glyceraldehyde groups and the β-1 or diarylpropane units have been found in lignin preparations, but it has proved difficult to estimate the abundance of such structures in lignin. Degradation products emanating from the β-1 structure are prominent among thioacidolysis products,[5] whereas NMR studies on milled wood lignin have revealed only small amounts of such structures.[6–8] It is still not known if this discrepancy is caused by an uneven distribution of such structures in lignins or if the explanation is that the cyclohexadienone structures persist in the lignin and that they are transformed to β-1 structures only on acidic treatment. The likelihood that such structures can persist in lignin is increased by the recent discovery in nature of woorenol,[9] a neolignan with a β-1 coupled cyclohexadienone structure. Recent isolation of isochroman structures in lignin degradation products and their detection in isolated lignins provides evidence for such spiro structures *in vivo*;[10,11] in this case a dienone–phenol rearrangement may have produced an isochroman structure. We have now been able to generate a spirocyclohexadienone structure similar to that in woorenol from a biomimetic oxidative phenol coupling and to demonstrate that mild acidolysis indeed transforms it to a 1,2-diarylpropane-1,3-diol structure; this constitutes the first *in vitro* demonstration of the original mechanism put forward in 1965.[2]



Scheme 1

Results and discussion

We have reported earlier that cross coupling of phenols with different oxidation potentials is difficult to achieve. For

instance, sinapyl alcohol does not react with a syringyl structure with a saturated side chain.[1] The lower oxidation potential of the sinapyl alcohol compared to the syringyl structure makes the sinapyl alcohol react mainly with itself. Replacing the alcohol hydroxy with a carboxy group is expected to raise the oxidation potential of the phenol[12] to the level of the syringyl structure. This was, in fact, observed. Oxidative coupling (with hydrogen peroxide catalyzed by horseradish peroxidase) of an equimolar mixture of **1** with methyl sinapate (**2**) gave a product that could be assigned structure **3** on the basis of a crystal structure determination of the acetate (Fig. 1). Another prominent product was **4**, a dimerization product of methyl sinapate,[13] analogous to a ferulate dimer observed in grass cell walls.[14] The spiro cyclohexadienone structure of **3** can be visualized as having been formed by a β-1 coupling between **1** and **2** and subsequent cyclization (Scheme 2). Mild acid hydrolysis converted this cyclohexadienone to **5**, the first demonstration of a β-1 structure formed *in vitro* by oxidative coupling and side chain elimination. The 1,2-diarylpropane **5** was obtained as a single diastereomer; the stereochemistry of the reaction is under investigation. The β-1 pattern is a minor contribution to the structure of lignin but knowledge about its mode of formation and reactivity sheds important light on the factors regulating the biosynthesis of lignin in the cell wall of the plant.

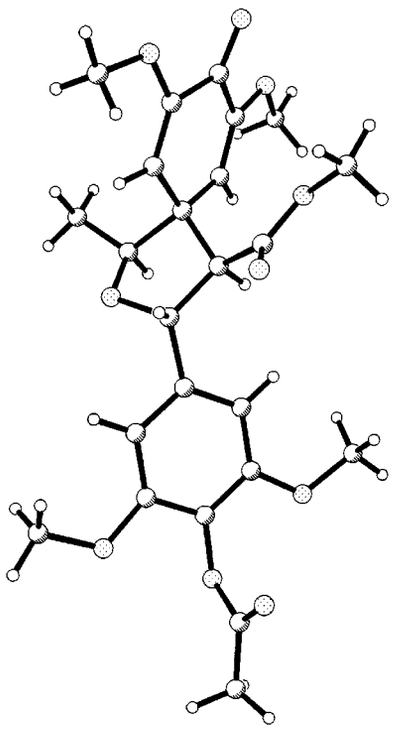
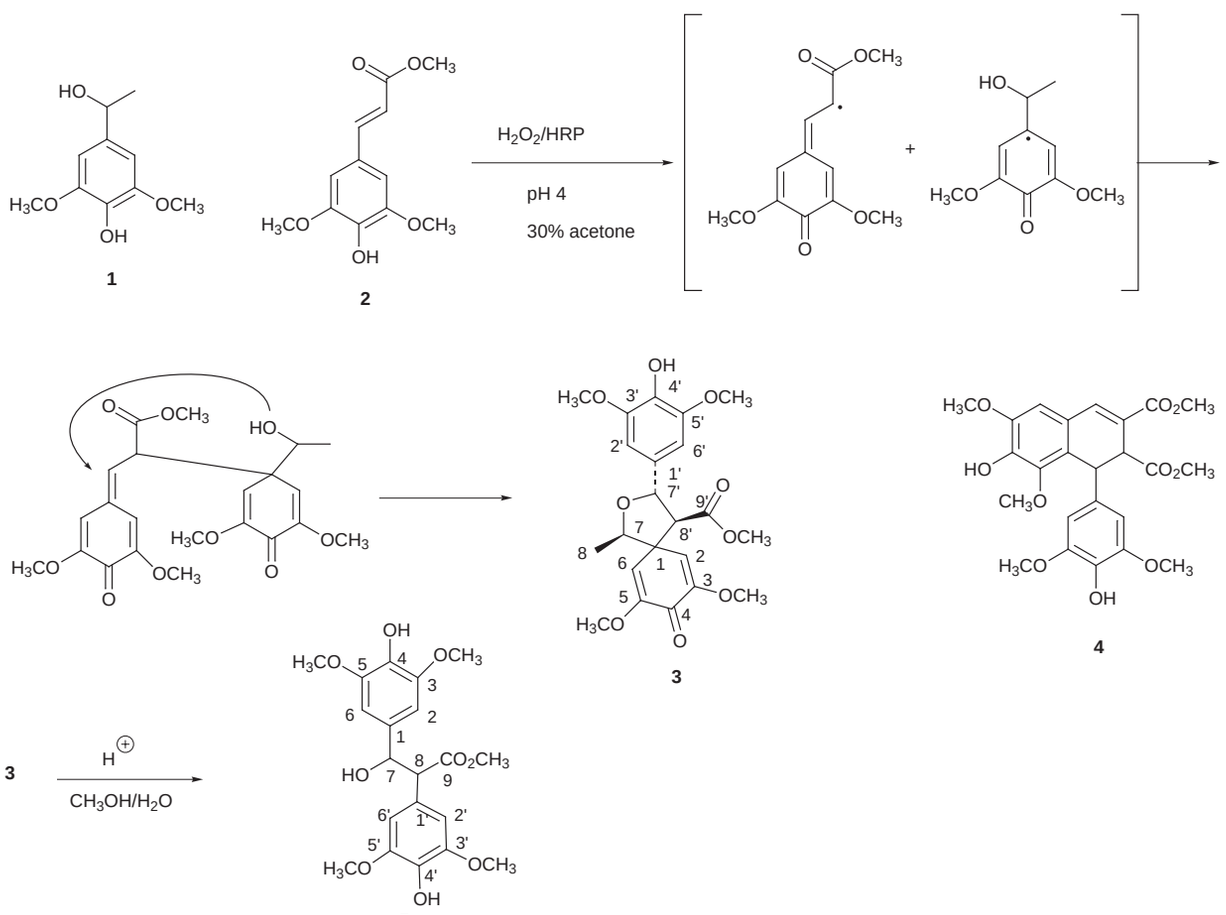


Fig. 1 Crystal structure of the acetate of **3**.

Experimental

General

Horseradish peroxidase (EC 1.11.1.7) was from Serva, activity 250 U mg^{-1} (purpurogallin method). 30% Aqueous hydrogen peroxide (Merck) was diluted to give a 3% solution (*ca*. 0.8 mmol cm^{-3}) before use. Silica gel for column chromatography was Merck 60 (230–400 mesh). Thin layer chromatography was performed on silica gel plates (Merck Kieselgel 60 F_{254}). The preparative HPLC was performed with detection at 265 nm and a Lichrospher Si 60 column (1 × 25 cm) from Merck. The injection volume was 1 cm^3. ^1H and ^{13}C NMR spectra were recorded with Varian 200 (2000 Gemini) and 300 MHz (Nova) instruments with tetramethylsilane (TMS) as internal standard and



Scheme 2

deuteriochloroform as solvent. The spectral assignments were made using HMQC-TOCSY and HMBC experiments and VNMR software. Mass spectra were recorded with a JEOL JMS-SX102 instrument. The melting points are uncorrected.

1-(4-Hydroxy-3,5-dimethoxyphenyl)ethanol 1

This compound was prepared from syringaldehyde and methyl magnesium iodide.[15]

Methyl (*E*)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoate 2 (methyl (*E*)-sinapate)

The compound was made from vanillin. Bromination and methoxylation gave syringaldehyde.[16] Knoevenagel reaction with malonic acid[17] and esterification with methanol–sulfuric acid gave the title compound.

Oxidative coupling of 1 and 2

Methyl sinapate (**2**) (0.79 g, 3.33 mmol) and **1** (0.66 g, 3.33 mmol) were dissolved in acetone (30 ml) and a solution of buffer (0.02 mol dm^{-3}, citrate–phosphate, pH 3.5; 75 ml) was added. Horseradish peroxidase (40 mg in 1 ml of water) was added and hydrogen peroxide (4.16 ml, 3.33 mmol) was added during 10 minutes to the reaction mixture which was stirred at room temperature for 2 hours. The mixture was extracted three times with ethyl acetate and the combined extracts were washed with 5% aqueous sodium hydrogen carbonate, water and brine, dried (Na_2SO_4) and evaporated. The residue was acetylated with acetic anhydride and dry pyridine (1 : 1) overnight at room temperature.[18] The dark brown mixture was first purified using a short silica gel 60 column (4 × 5 cm) eluting with ethyl acetate–hexane (3 : 1). The fractions containing the products (detected with TLC) were combined and evaporated to a yellowish powder. The crude product was then separated using preparative HPLC (eluent: ethyl acetate–hexane, 3 : 1) to yield 298 mg (19%) of **3**, a large amount (*ca*. 50%) of unreacted **1** and a small amount (*ca*. 4%) of sinapate dimer **4**.[13] Compound **3** was recrystallized for crystal structure determination from ethyl acetate–hexane, mp 170 °C (decomp.).

Methyl 3-hydroxy-2,3-bis(4-hydroxy-3,5-dimethoxyphenyl)-propanoate 5

Compound **3** (80 mg, 0.163 mmol) in methanol (10 ml) was treated with toluene-*p*-sulfonic acid monohydrate (3 mg) at room temperature for 20 hours. The reaction mixture was neutralized by treating with solid sodium hydrogen carbonate, 5 ml of water was added and the mixture extracted with dichloromethane. The organic phase was washed with water and with brine, dried (Na_2SO_4) and evaporated. The crude product was acetylated with acetic anhydride (2 ml) and pyridine (2 ml). The acetylated reaction mixture was dissolved in ethyl acetate. On dissolution, part of compound **5** (peracetate) started to crystallize and the crystals were filtered off. The rest was isolated by flash chromatography on a silica column with a 2 : 1 mixture of ethyl acetate and hexane. The combined yield of **5** (peracetate) was 30 mg (33%), white crystals mp 210–212 °C. NMR data in Table 1; MS: *m*/*z* 534 (M^+, 15%), 492 (30), 432 (16), 390 (36), 268 (65), 225 (100), 197 (15), 183 (93) (Found: M^+, 534.1743. $C_{26}H_{30}O_{12}$ requires *M*, 534.1737).

Crystal structure of 3 acetate

Crystal data. $C_{24}H_{28}O_{10}$, $M = 476.46$. Monoclinic, $a = 9.133(2)$, $b = 10.921(2)$, $c = 22.979(5)$ Å. $\beta = 91.29(3)°$, $V = 2291.4(8)$ $Å^3$ (by least-squares fitting of 20 automatically centred reflections); $F(000) = 1008$, $D_x = 1.38$ g cm^{-3}, space group $P2_1/c$, $Z = 4$, μ(Mo-Kα) = 0.108 mm^{-1}. Colourless plates, 0.40 × 0.37 × 0.32 mm, recrystallized from ethyl acetate–hexane.

Table 1 ^1H and ^{13}C NMR data for acetates of compounds **3** and **5**

	3		**5**	
Position	^{13}C	^1H	^{13}C	^1H
1	52.1		136.2	
2	114.7	5.89 (d, *J* 2)	104.2	6.64 (s)
3	152.6		152.0	
4	176.0		132.9	
5	152.1		152.0	
6	111.3	5.61 (d, *J* 2)	104.2	6.64 (s)
7	83.5	4.41 (d, *J* 6.10)	74.9	6.42 (d, *J* 9.5)
8	14.7	1.06 (d, *J* 6.10)	57.7	3.99 (d, *J* 9.5)
3 OMe	55.1	3.61	56.3	3.82
5 OMe	54.4	3.65	56.3	3.82
1′	140.2		136.2	
2′	101.0	6.55 (s)	105.7	6.62 (s)
3′	152.0		152.0	
4′	127.7		132.9	
5′	152.0		152.0	
6′	101.0	6.55 (s)	105.7	6.62 (s)
7′	79.7	5.65 (d, *J* 8.8)		
8′	63.1	3.38 (d, *J* 8.8)		
9′	169.2			
4 O*C*O			168.6	
4 OCOMe			20.5	2.33 (s)
4′ O*C*O	168.4		168.6	
4′ OCOMe	20.1	2.24 (s)	20.5	2.33 (s)
3′,5′ OMe	55.9	3.74 (s)	56.3	3.82 (s)
7 O*C*O			169.4	
7 OCOMe			20.8	1.87 (s)
9′ OMe	54.2	3.47 (s)		
9 OCO			170.8	
9 OMe			52.3	3.56 (s)

Data collection and processing. Data were collected on a Rigaku AFC7S diffractometer using graphite monochromated Mo-Kα radiation at −80 °C. Three standard reflections were monitored every 2 h and showed no significant deviation. 3165 Unique reflections were recorded ($2.57 < \theta < 25.01°$ ±*h*,*k*,*l*).

Structure analysis and refinement. Positional parameters were determined by direct methods and the non-hydrogen atoms were refined by full-matrix least-squares on F^2. The hydrogen atoms were placed at calculated positions and were assigned isotropic thermal parameters $U(H) = 1.5\ U_{eq}(C)$ of the parent carbon atoms. The refinement converged at $R_1 = 0.0762$ {1774 $I > 2\sigma(I)$ reflections} and $wR_2 = 0.2004$ for all reflections. The final electron difference map was featureless with largest peak 0.62 e $Å^{-3}$. Computations were carried out on using the SHELXTL[19] and SHELXL93[20] program systems.

Full crystallographic details, excluding structure factor tables, have been deposited at the Cambridge Crystallographic Data Centre (CCDC). For details of the deposition scheme, see 'Instructions for Authors', *J. Chem. Soc*., *Perkin Trans. 1*, available *via* the RSC Web page (http://www.rsc.org/authors). Any request to the CCDC for this material should quote the full literature citation and the reference number 207/293. See http://www.rsc.org/suppdata/p1/1999/461/ for crystallographic files in .cif format.

References

1 K. Syrjänen and G. Brunow, *J. Chem. Soc.*, *Perkin Trans. 1*, 1998, 3425.
2 K. Lundquist and G. E. Miksche, *Tetrahedron Lett.*, 1965, 2131.
3 H. Nimz, *Chem. Ber.*, 1965, **98**, 3160.
4 K. Freudenberg and A. C. Neish, *Constitution and Biosynthesis of Lignin*, Springer-Verlag, Heidelberg, 1968, Vol. 2.
5 C. Lapierre, B. Pollet and B. Monties, *Phytochemistry*, 1991, **30**, 659.
6 K. Lundquist, *J. Wood Chem. Technol.*, 1987, **7**, 179.
7 I. Kilpeläinen, J. Sipilä, G. Brunow, K. Lundquist and R. M. Ede, *J. Agric. Food Chem.*, 1994, **42**, 2790.
8 G. Brunow, E. Ämmälahti, T. Niemi, J. Sipilä, L. K. Simola and K. Ilkka, *Phytochemistry*, 1998, **47**, 1495.

9 K. Yoshikawa, H. Kinoshita and S. Arihara, *J. Nat. Prod.*, 1997, **60**, 511.
10 J. Ralph, J. Peng and F. Lu, *Tetrahedron Lett.*, 1998, **39**, 4963.
11 J. Peng, F. Lu and J. Ralph, *Phytochemistry*, 1998, **50**, 659.
12 M. Jonsson, J. Lind, T. Reitberger, T. E. Eriksen and G. Merényi, *J. Phys. Chem.*, 1993, **97**, 8229.
13 H. Setälä, A. Pajunen, I. Kilpeläinen and G. Brunow, *J. Chem. Soc.*, *Perkin Trans. 1*, 1994, 1163.
14 J. Ralph, S. Quideau, J. H. Grabber and R. D. Hatfield, *J. Chem. Soc.*, *Perkin Trans. 1*, 1994, 3485.
15 J. Pospisek, M. Pisova and M. Soucek, *Collect. Czech. Chem. Commun.*, 1975, **40**, 142.
16 P. S. Manchand, P. S. Belica and H. S. Wong, *Synth. Commun.*, 1990, **20**, 2659.
17 K. Freudenberg and H. H. Hübner, *Chem. Ber.*, 1952, **85**, 1181.
18 E. Adler, G. Brunow and K. Lundquist, *Holzforschung*, 1987, **41**, 199.
19 G. M. Sheldrick, *SHELXTL/PC. Users Manual, Siemens Analytical X-ray Instruments Inc.*, Madison, WI, USA, 1990.
20 G. M. Sheldrick *SHELX93. Program for the Refinement of Crystal Structures*, 1993.

Paper 8/08884E