Horse Radish Peroxidase-catalysed Oxidative Coupling of Methyl Sinapate to give Diastereoisomeric Spiro Dimers

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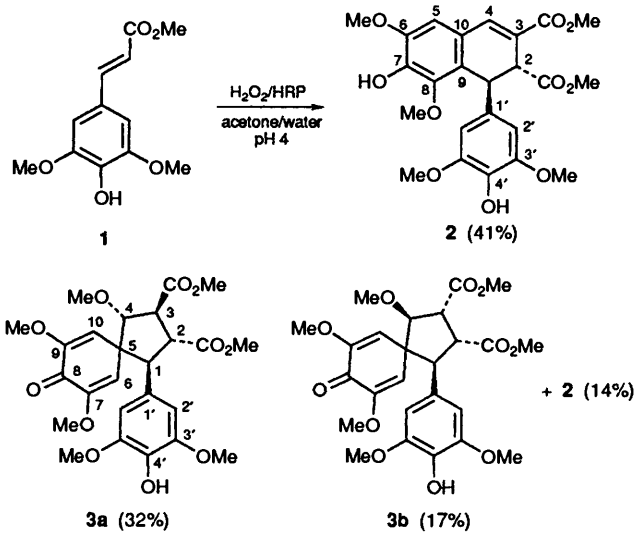
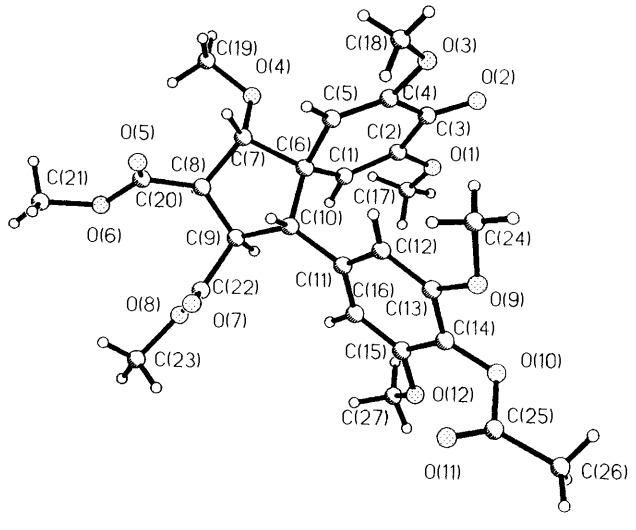
The oxidative coupling of methyl sinapate with H_2O_2/horse radish peroxidase at pH 4 in the presence of methanol gives dimeric spiro structures of a novel type. The crystal structures of two diastereoisomers have been determined. The coupling reaction shows some *threo* selectivity.

Aryltetralins are an important group of natural lignans with a 1,2-dihydro- or tetrahydro-naphthalene skeleton. Many of these compounds have been observed to have biological and pharmacological effects.[1] The oxidative coupling of phenols catalyzed by peroxidases is a very attractive method for preparing phenolic dimers of this type from phenolic cinnamates. The advantages of the enzymatic method are mild reaction conditions and fast reaction rates. The use of peroxidases in a preparatively useful manner is limited by the low selectivity of the oxidative coupling and the complexity of the subsequent reactions. We have been investigating possible ways to enhance the selectivity of this reaction type, for example, by changing the pH or using organic co-solvents.[2,3] In this paper we report how the addition of methanol changes the course of the oxidative dimerization of (*E*)-methyl sinapate giving two diastereoisomers of an interesting new spiro compound.

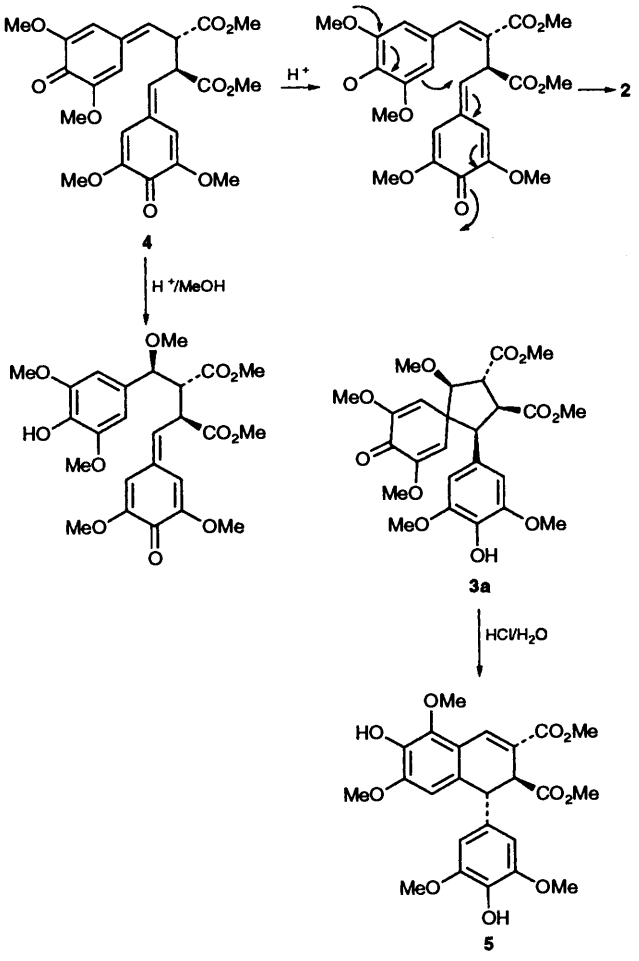
In a previous investigation of the oxidative coupling of methyl sinapate published by Wallis,[4] the reaction was carried out with ferric chloride in aqueous acetone. In our work the oxidant was hydrogen peroxide with horse radish peroxidase, HRP (EC 1.11.1.7), as a catalyst. The reactions were carried out at pH 4 because our previous work with ferulates have shown that low pH-values tend to favour the formation of dimers at the expense of polymeric products.[3] When the reaction was carried out in aqueous acetone, compound **2** was observed as the only dimeric product (yield 41%) after acidification. Repeating the oxidation in aqueous methanol, compound **2** was a minor product, the main products being the diastereoisomeric spiro compounds **3a** and **3b** (Scheme 1). The structures of **3a** and **3b** were determined by X-ray crystallography. The formation of compound **2** was also observed in the work of Wallis and its formation can be understood as a prototropic rearrangement of an initially formed bisquinone methide **4** to a cinnamyl structure and subsequent cyclization of the other quinone methide onto the aromatic ring (Scheme 2).[4] In formation of the spiro compounds **3a** and **3b**, we assume that the addition of methanol to the bisquinone methide **4** is a key step. We have previously observed that methanol at pH 4 reacts rapidly with quinone methides, even in aqueous solvents.[3] The next step can be formulated as an electrophilic attack by the protonated quinone methide on the aromatic ring to form the spiro compound (Scheme 2). Since it is unclear why five-membered ring formation is favoured in this case, the possible participation of phenoxy radicals cannot be ruled out. A possible mechanism where intermediate spiro compounds would result in the formation of **2** was ruled out by carrying out an acid hydrolysis of a mixture of **3a** and **3b**. In the dienone–phenol rearrangement only the oxygen-substituted side-chain migrated to give the isomeric dihydronaphthol **5** after elimination of methanol.



Scheme 1



The structure of the diastereoisomers **3a** and **3b** were determined by X-ray crystallography of the acetates and the assignments of the signals in the ^1H and ^{13}C NMR spectra were carried out using 2D NMR techniques (HMBC and HMQC). The *trans* configuration of the ester groups in the predominant dimer **3a** shows that the radical coupling leading to the β–β bond has a stereospecificity consistent with the *threo* coupling observed with other 4-hydroxyphenylpropenes.[5]



Scheme 2

Experimental

General.—Horse radish peroxidase (EC 1.11.1.7) was from Serva, activity 277 U mg^{-1} (purpurogallin method). Hydrogen peroxide, a 30% aqueous solution from Merck, was diluted to give a 3% solution before use. Silica gel 60 (0.040–0.063) for flash chromatography was from Merck. The preparative HPLC was performed with detection at 265 nm and a column (1 × 30 cm) with Silasorb 600 as an absorbent. Hexane–ethyl acetate was used as an eluent. The injection volume was 1 cm^3. ^1H and ^{13}C NMR measurements were recorded with a Varian Unity 500 spectrometer with tetramethylsilane as internal standard. MS were recorded with a JEOL JMS-SX102 instrument. The melting points are uncorrected.

(E)-*Methyl Sinapate* (*Methyl* 4-*Hydroxy*-3,5-*dimethoxybenzylideneacetate*) **1**.—This compound was synthesised from vanillin. Bromination and methoxylation gave 5-methoxyvanillin (syringaldehyde).[6] A subsequent Knoevenagel reaction with malonic acid[7] and esterification with methanol–sulfuric acid gave the title compound.

Oxidative Coupling of (E)-*Methyl Sinapate in Aqueous Acetone.—Procedure* (*A*). Methyl sinapate (0.40 g, 1.68 mmol) was dissolved in acetone (10 cm^3) and a solution of buffer (0.02 mol dm^{-3}, citrate–phosphate, pH 4; 40 cm^3) was added to it. Hydrogen peroxide (0.85 cm^3, 0.84 mmol) and aqueous HRP (1400 U; 1 cm^3) were added during 10 min to the reaction mixture which was then stirred at room temperature for 1 h during which time it turned reddish brown. HCl (2 mol dm^{-3}; 5 cm^3) was added to the reaction mixture which, whilst being stirred for 20 min at room temperature, turned yellowish. The mixture was extracted with ethyl acetate (× 3) and the combined extracts were washed with 5% aqueous $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated to dryness. The residue was acetylated with dry pyridine and acetic anhydride (1:1) overnight at room temperature.[8] The products were separated using a short silica gel column (3 × 4 cm) eluting with hexane–AcOEt (1:1). The main fraction (351 mg) was purified by preparative HPLC (eluent: hexane–AcOEt, 1:1) to yield *dimethyl* 7-*acetoxy*-1-(4-*acetoxy*-3,5-*dimethoxyphenyl*)-6,8-*dimethoxy*-1,2-*dihydronaphthalene*-2,3-*dicarboxylate* (diacetate of **2**) which recrystallized from ethyl acetate–hexane (1:4) as white crystals, m.p. 177–179 °C; δ_H(500 MHz; $CDCl_3$) 2.28 (3 H, s, 4'-$OCOCH_3$), 2.33 (3 H, s, 7-$OCOCH_3$), 3.64 (3 H, s, 8-OMe), 3.66 (3 H, s, 2-CO_2CH_3), 3.68 (6 H, s, 3',5'-OMe), 3.80 (3 H, s, 3-CO_2CH_3), 3.86 (3 H, s, 6-OMe), 4.17 (1 H, d, *J* 1.1, 2-H), 5.03 (1 H, s, 1-H), 6.23 (2 H, s, 2',6'-H), 6.76 (1 H, s, 5-H), 7.62 (1 H, s, 4-H); δ_C 20.5 ($OCOCH_3$), 39.3 (1-C), 45.6 (2-C), 52.1 (3-CO_2CH_3), 52.6 (2-CO_2CH_3), 56.1 (3',5'-OMe), 56.2 (6-OMe), 61.4 (8-OMe), 104.1 (2',6'-C), 108 (5-C), 122.7 (9-C), 125.7 (3-C), 127.5 (4'-C), 129.8 (1'-C), 135.0 (7-C), 136.7 (4-C), 140.4 (10-C), 151.0 (8-C), 151.9 (6-C), 166.7 (3-CO_2CH_3), 168.1 (7-$OCOCH_3$), 168.7 (4'-$OCOCH_3$) and 172.0 (2-CO_2CH_3); *m/z* 558 (M^+, 24%), 516 (98), 499 (10), 474 (69), 456 (15), 414 (100), 383 (37), 355 (12), 320 (16), 289 (10) and 236 (10) (Found: M^+, 558.1735. $C_{28}H_{30}O_{12}$ requires *M*, 558.1737).

Further elution yielded a mixture of by-products (70 mg, 15%) and approximately 44% of the total yield was oligomeric material eluted from the silica gel column with EtOH–AcOEt (1:1).

Procedure (*B*). The reaction was performed in a similar way without the addition of HCl. The reddish brown reaction mixture turned slowly yellowish over 12 h. After this time the same work-up yielded the dimer **2** (152 mg, 32%).

Oxidative Coupling of (E)-*Methyl Sinapate in Aqueous Methanol.*—Methyl sinapate (1.44 g, 6.05 mmol) was dissolved in methanol (200 cm^3) and a solution of buffer (0.02 mol dm^{-3}, citrate-phosphate, pH 4; 450 cm^3) was added to it. Hydrogen peroxide (3.84 cm^3, 3.03 mmol) and aqueous HRP (6066 U, 10 cm^3) were added during 15 min to the reaction mixture which was then stirred for 2 h at room temperature. The cloudy, yellowish reaction mixture was extracted several times with ethyl acetate and the compound extracts were washed with 5% aqueous sodium hydrogen carbonate, water and brine, dried (Na_2SO_4) and evaporated to dryness. The products were acetylated as above.

Separation and Purification of the Products.—The acetates of **2** and **3** were separated by preparative HPLC with hexane–ethyl acetate (1:4) as eluent. The yield of dimer **2** was 238 mg (14%). The fraction containing the diastereoisomers **3a** and **3b** was then further fractionated with hexane–ethyl acetate (1:9).

Dimethyl 1-(4-*Acetoxy*-3,5-*dimethoxyphenyl*)-4,7,9-*trimethoxy*-8-*oxospiro*[4.5]*deca*-6,9-*diene*-2,3-*dicarboxylate* (*Acetate of* **3**).—The yield of the acetates of **3** after HPLC was 49% including two diastereoisomers **3a** (32%) and **3b** (17%). Both of these were crystallised from diethyl ether–acetone mixture (9:1), **3a** m.p. 195–196 °C and **3b** m.p. 183–185 °C; ^1H NMR and ^{13}C NMR (**3a** in [$^2H_6$]acetone) are shown in Table 1: **3a**, *m/z* 548 (M^+, 4%), 516 (42), 474 (25), 456 (49), 414 (100), 383 (31), 372 (26), 355 (18), 336 (23) and 170 (16) (Found: M^+, 548.1901. $C_{27}H_{32}O_{12}$ requires *M*, 548.1894); **3b**, *m/z* 548 (M^+, 30%), 516 (83), 474 (58), 456 (26), 414 (100), 383 (40), 355 (13), 320 (15), 268 (17) and 170 (16) (Found: M^+, 548.1904. $C_{27}H_{32}O_{12}$ requires *M*, 548.1894).

Table 1 The assignment of ^1H and ^{13}C chemical shifts for acetates of compounds **3a** and **3b** (500 MHz, solvent [$^2H_6$]acetone, *J* values are given in Hz)

	3a		**3b**	
Assignment	δ_H	δ_C	δ_H	δ_C
4'-OAc	2.16 (s)	20.23	2.16 (s)	20.21
4-OCH_3	3.38 (s)	58.63	3.27 (s)	59.91
3-CH	3.48 (dd, *J* 8.8 and 3.6)	54.90	3.60 (dd, *J* 12.4 and 9.2	50.99
9-OCH_3	3.57 (s)	55.40	3.68 (s)	55.64
3-CO_2CH_3	3.60 (s)	52.49	3.71 (s)	52.49
7-OCH_3	3.72 (s)	55.47	3.75 (s)	55.56
3'-, 5'-OCH_3	3.74 (s)	56.50	3.73 (s)	56.44
2-CO_2CH_3	3.79 (s)	52.94	3.56 (s)	52.34
1-CH	3.94 (d, *J* 12.1)	58.57	3.88 (d, *J* 11.9)	56.50
4-CH	4.01 (d, *J* 3.4)	91.44	4.48 (d, *J* 9.3)	91.24
2-CH	4.04 (dd, *J* 12.0 and 8.8)	49.09	4.33 (br d, *J* 12.1)	47.15
10-CH	6.04 (d, *J* 2.4)	114.81	6.31 (d, *J* 2.4)	113.32
6-CH	6.28 (d, *J* 2.4)	117.09	6.42 (d, *J* 2.4)	120.13
2'-, 6'-CH	6.64 (s)	106.41	6.65 (s)	106.51
5-C (spiro)		55.20		55.40
4'-C		128.90		128.88
1'-C		135.18		135.28
9-C		152.24		153.76
3'-, 5'-C		152.36		152.29
7-C		152.49		153.18
2-CO_2CH_3		173.66		172.97
3-CO_2CH_3		173.91		173.43
8-C=O		175.47		175.98

Dimethyl 6-Acetoxy-1-(4-acetoxy-3,5-dimethoxyphenyl)-5,7-dimethoxy-1,2-dihydronaphthalene-2,3-dicarboxylate (Acetate of **5**).—Compound **3** (100 mg, 0.18 mmol) was dissolved in dioxane (9 cm^3) and 0.2 mol dm^{-3} HCl (1 cm^3) was added to the solution. The mixture was refluxed for 30 min after which it was extracted with ethyl acetate (2 × 50 cm^3). The combined extracts were washed with aqueous sodium hydrogen carbonate, water and brine, dried (Na_2SO_4) and evaporated to dryness. The residue was acetylated and purified by preparative HPLC with hexane–ethyl acetate (1 : 1) as eluent to give the diacetate of **5** as white crystals (EtOH), m.p. 186–187 °C; δ_H(500 MHz) 2.30 (3 H, s, 4'-$OCOCH_3$), 2.36 (3 H, s, 6-$OCOCH_3$), 3.66 (3 H, s, 2-CO_2CH_3), 3.69 (6 H, s, 3',5'-OCH_3), 3.78 (3 H, s, 3-CO_2CH_3), 3.79 (3 H, s, 7-OCH_3), 3.88 (3 H, s, 5-OCH_3), 4.04 (1 H, d, *J* 2.6, 2-H), 4.68 (1 H, d, *J* 2.6, 1-H), 6.23 (2 H, s, 2',6'-H), 6.53 (1 H, s, 8-H) and 7.94 (1 H, s, 4-H); δ_C 20.5 (4',6-$OCOCH_3$), 46.8 (C-1), 46.9 (C-2), 52.0 (3-CO_2CH_3), 52.6 (2-CO_2CH_3), 56.1 (3',5'-OCH_3), 56.2 (7-OCH_3), 62.4 (5-OCH_3), 104.4 (C-2',6'), 108.5 (C-8), 118.6 (C-10), 122.6 (C-3), 127.6 (C-4'), 131.1 (C-4), 132.1 (C-6), 135.5 (C-9), 140.2 (C-1'), 150.9 (C-5), 154.0 (C-7), 152.0 (C-3',5'), 166.9 (3-CO_2CH_3), 168.4 (6-$OCOCH_3$), 168.8 (4'-$OCOCH_3$), 172.5 (2-CO_2CH_3); *m/z* 558 (M^+, 49%), 516 (55), 499 (12), 474 (10), 456 (51), 414 (100), 383 (25), 320 (13), 195 (11) and 167 (13) (Found: M^+, 558.1741. $C_{28}H_{30}O_{12}$ requires *M*, 558.1737).

Crystal Structure of **3b** *Acetate.—Crystal data.* $C_{27}H_{32}O_{12}$, M_r = 548.54. Monoclinic, *a* = 11.818(5), *b* = 23.828(5), *c* = 11.016(5) Å, β = 107.12(5)°, *V* = 2965(2) $Å^3$ (by least-squares refinement on diffractometer angles of 16 automatically centered reflections); *F*(000) = 1160, D_x = 1.23 g cm^{-3}, space group $P2_1$, *Z* = 4, μ(Mo-Kα) = 0.097 mm^{-1}, (Mo-Kα) = 0.71069 Å. White plates, recrystallized from methanol.

Data collection and processing. Data were collected on a Nicolet P3 diffractometer using graphite monochromated Mo-Kα radiation and ω-2θ scan type. Two standard reflections were monitored every 2 h and showed no significant deviation. 2874 Unique reflections were recorded ($1.5 < \theta < 20°$, $\pm h,k,l$) but owing to the very poor quality of crystals only 1755 [$F > 5.0\sigma(F)$] were used in the refinement.

Structure analysis and refinement. Positional parameters were determined by direct methods using SHELXTL,[9] and were refined by full-matrix least-squares calculations in two blocks with all non-hydrogen atoms treated anisotropically using the weighting scheme $w^{-1} = \sigma^2(F) + 0.0376F^2$. The hydrogen atoms were placed in calculated positions. The atomic scattering factors were those in the SHELXTL program. The refinement converged at R = 0.135 (R_w = 0.157). Fractional atomic coordinates, bond lengths, angles and H-atom coordinates have been deposited at the Cambridge Crystallographic Data Centre.*

The crystal structure of **3a** will be published separately.

* For details see 'Instructions for Authors (1994),' *J. Chem. Soc., Perkin Trans. 1*, 1994, Issue 1.

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