Enzyme-mediated H_2O_2 Oxidation of (*E*)-Stilbene-3,4-diol

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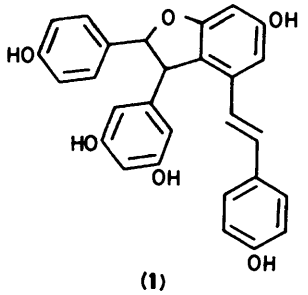
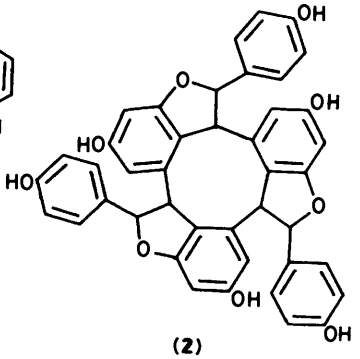
Enzyme-mediated H_2O_2 oxidation of (*E*)-stilbene-3,4-diol gave two dimeric compounds. The major product proved to be a 2,3-dihydro-1,4-benzodioxin (**5a**) the structure of which was established by *X*-ray analysis of its diacetate (**5b**).

The role of stilbenes in the wood pulping industry and in the discolouration of woods has led to studies of their oxidation reactions. Currently, stilbenes are receiving attention as possible phytoalexins.

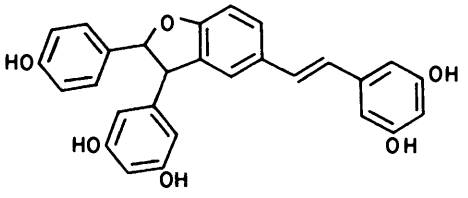
Resveratrol (stilbene-3,4′,5-triol) and its oligomers were isolated from the grapevine (*Vitis vinifera*)[1] and from the groundnut (*Arachis hypogaea*).[2] This stilbene was observed to accumulate in the detached leaves of the grapevine after infection with *Botrytis cinerea*.[1] Co-occurring in the leaf extract were the dimer ε-viniferin (**1**), the cyclic trimer α-viniferin (**2**), and a tetramer γ-viniferin. Of these oligomers, α-viniferin (**2**) was found to be the most active antifungal component. The viniferins were thought to arise *via* oxidation of resveratrol and by a route similar to that of the formation of the dimeric phenylpropanoid lignans.[3]



(**1**)



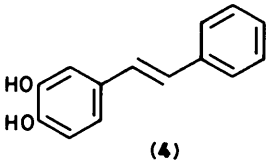
(**2**)



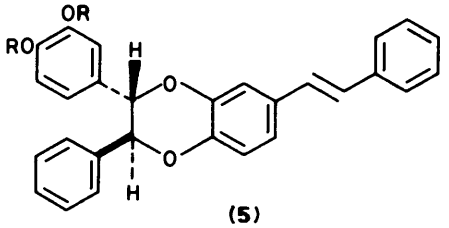
(**3**)

The oxidative dimerisation of some natural stilbenes were reported to give dihydrobenzofuran dimers.[4] The dimer (**3**) isolated from oxidation of resveratrol had similar biological properties to those of ε-viniferin (**1**).[1,5]

Here we report our investigations on the enzyme-mediated (horseradish peroxidase) H_2O_2 oxidation of (*E*)-stilbene-3,4-diol (**4**) which yielded two dimeric products (**5a**) and (**6a**). The

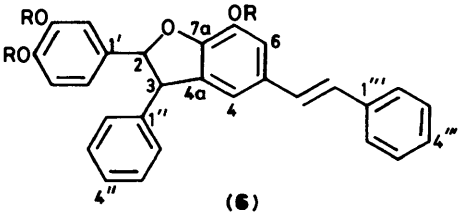


(**4**)



(**5**)

a; R = H
b; R = Ac



(**6**)

a; R = H
b; R = Ac

major product (**5a**) was characterised as its crystalline diacetate ($C_{32}H_{26}O_6$) (**5b**). In the mass spectrum of the diacetate the fragmentation pattern was identical with that of the parent stilbene. The formation of the fragment ions could be explained by processes involving ring opening of a 1,4-dioxane, the main fragment being the disubstituted ethylene molecule.[6] The structure (**5b**) was supported also by its u.v. spectrum ($\lambda_{max.}$ 302 and 317 nm) and i.r. spectrum [$\nu_{max.}$ 1 778, 1 776 (OAc) and 1 591, 971 cm^{-1} (*trans* double bond)]. The ^1H n.m.r. (300 MHz) showed the presence of (i) two acetate methyl groups δ_H 2.27 and 2.28, (ii) two *trans*-coupled protons δ_H 4.88 (1 H, d, *J* 8.1 Hz) and 4.94 (1 H, d, *J* 8.1 Hz), and (iii) overlapping multiplets in the

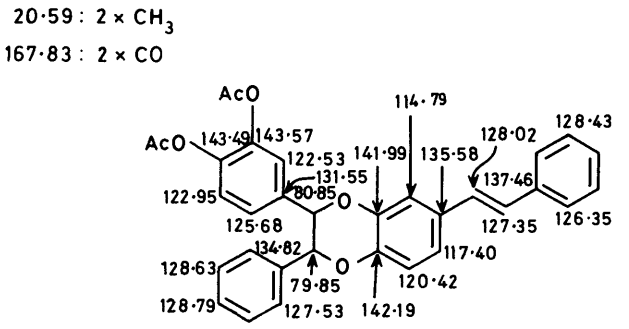


Figure 1.

aromatic region integrating for 18H. The off-resonance ^{13}C n.m.r. (300 MHz) had four singlets (δ_C 141—143 p.p.m.) for four phenolic carbon atoms, twelve doublets (δ_C 114—128 p.p.m.) for aromatic protons, two doublets (δ_C 127.3 and 128.0 p.p.m.) for olefinic protons, two further doublets (δ_C 79.85 and 80.85 p.p.m.) for aliphatic oxygen bearing carbon atoms, and four singlets (δ_C 131—137 p.p.m.) for quarternary carbons. In addition, singlets at 167.83 and 20.59 p.p.m. for the carbonyl and methyl carbons of the acetyl groups are present. The ^{13}C n.m.r. chemical-shift assignments are shown in Figure 1.

Unequivocal proof for the structure of compound (**5a**) was provided by single-crystal *X*-ray analysis of its diacetate (**5b**).

X-*Ray Structure Determination of Compound* (**5b**).—A crystal of (**5b**) grown from acetone was mounted on a four circle automatic Phillips PW 1100 diffractometer using a graphite monochromator with Cu-K_A radiation (λ = 1.5418 Å). The system is monoclinic, space group $P2_1/c$ with four molecules in the cell. The parameters are: a = 15.351(3), b = 9.249(2), c = 19.011(3) Å and β = 101.6(5)° with a total volume of 2 644.3 Å ^{3}. Among a total of 4 645 recorded reflections (up to θ = 64°) 2 248 were found above the 3σ background level.

The structure was solved by the use of the multisolution technique.[7] Most of the atomic positions were found in the *E* map corresponding to the highest figure of merit; half of a phenyl ring and an acetoxy group were missing. The rest of the structure was obtained from Fourier recycling procedures. The refinements were performed by least-squares procedures with anisotropic thermal factors for the carbon and oxygen atoms. Hydrogen atoms were placed at their theoretical positions. The final *R* value was 0.061 with 2 518 structure factors (at 2.5σ) included in the last cycle. The view of the molecule is given in Figure 2.

Atomic positional parameters for the non-hydrogen atoms appear in Table 1, bond lengths are in Table 2. Positional and isotropic thermal parameters for the hydrogen atoms, and anisotropic thermal factors for the non-hydrogen atoms are available on request from the Cambridge Crystallographic Data Centre.*

Thus the major oxidation product is 2,3-dihydro-3-(3,4-dihydroxyphenyl)-2-phenyl-6-(2-phenylvinyl)-1,4-benzodioxin.

The benzodioxin structure (**5a**) presumably arises through a radical coupling mode (M_β + Mo)† to give a quinone-methide intermediate (**7**). Cyclisation by nucleophilic attack of the free phenolic group on the quinone-methide system would afford (**5a**) (Scheme).

A number of natural products possessing 1,4-benzodioxin structures have been reported. Compounds such as eusiderin-B (*Licaria rigida*)[8] and americanin (*Phytolacca americana*)[9] are

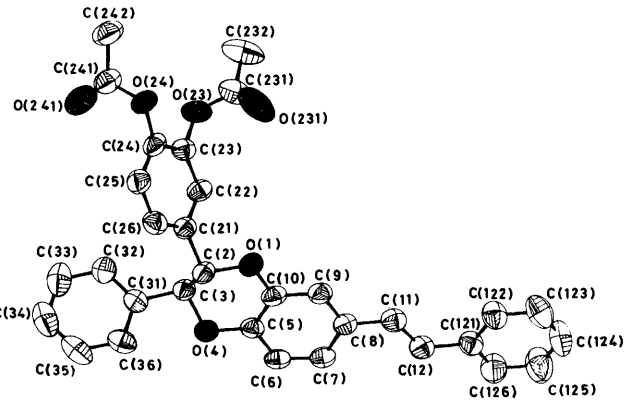
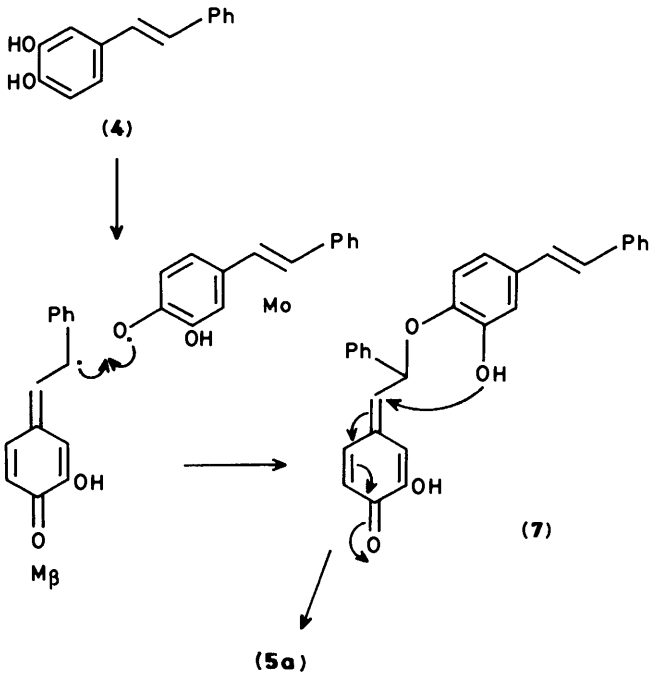


Figure 2. Perspective view of the diacetate (**5b**) showing crystallographic numbering scheme



Scheme.

thought to be the precursors of flavolignans. Recently, two new eusiderins-C and -D, found in the bark of *Virola pavonis*,[10] were allocated a *cis* stereochemistry. Other examples are the flavonoid silymarin, which has noted antihepatotoxic effect,[11] the cleomiscosins[12] and daphneticin.[13]

The minor product, dihydrobenzofuran (**6a**) was the result of coupling of M_β and M_5 mesomers.† Acetylation of compound (**6a**) with acetic anhydride and pyridine gave a triacetate (**6b**) as an oil, molecular formula $C_{34}H_{28}O_7$. U.v. data indicated the presence of an *E*-stilbene moiety. The i.r. spectrum had strong absorptions at 1 765 (CO), 1 730 (CO), and 1 595 cm^{-1} (aromatic C=C). The ^1H n.m.r. showed two doublets at δ_H 4.86 and 5.88 (1 H each, J 7.3 Hz) for protons on 3-C and 2-C respectively, and overlapping multiplets in the aromatic region integrating for 17H. The spectral data on (**6a**) compared well with the published results on similar structure types.[5]

The (*E*)-stilbene-3,4-diol used in the oxidation was prepared

* See Instructions for Authors (1987), *J. Chem. Soc., Perkin Trans. 1*, 1987, Issue 1.

† Coupling modes used in lignan chemistry.

Table 1. Positional parameters for the non hydrogen atoms ($\times 10^4$)

Atom	*x*	*y*	*z*
O(1)	4 012(2)	2 237(5)	4 959(2)
C(2)	3 725(3)	1 494(7)	4 281(3)
C(3)	4 252(3)	2 075(7)	3 738(3)
O(4)	5 191(2)	1 803(5)	4 012(2)
C(5)	5 489(4)	2 269(6)	4 711(3)
C(6)	6 392(4)	2 489(7)	4 962(3)
C(7)	6 712(4)	2 952(7)	5 651(3)
C(8)	6 136(4)	3 213(7)	6 115(3)
C(9)	5 235(4)	2 978(7)	5 865(3)
C(10)	4 916(4)	2 475(7)	5 171(3)
C(11)	6 440(4)	3 752(7)	6 855(3)
C(12)	7 285(4)	4 019(7)	7 167(3)
C(21)	2 741(4)	1 833(7)	4 067(3)
C(22)	2 459(4)	3 240(7)	4 162(3)
C(23)	1 549(4)	3 535(7)	3 992(3)
C(24)	947(4)	2 516(8)	3 739(3)
C(25)	1 214(4)	1 117(8)	3 625(3)
C(26)	2 130(4)	828(8)	3 795(3)
C(31)	4 024(4)	1 309(7)	3 022(3)
C(32)	4 418(5)	13(8)	2 905(4)
C(33)	4 185(6)	−651(9)	2 234(5)
C(34)	3 553(6)	−85(11)	1 703(5)
C(35)	3 167(6)	1 178(11)	1 818(4)
C(36)	3 389(5)	1 873(8)	2 475(3)
C(121)	7 607(4)	4 603(7)	7 902(3)
C(122)	8 468(4)	5 104(9)	8 084(4)
C(123)	8 805(5)	5 692(11)	8 744(5)
C(124)	8 287(6)	5 767(10)	9 250(4)
C(125)	7 419(6)	5 274(9)	9 077(4)
C(126)	7 082(4)	4 669(8)	8 416(3)
O(23)	1 264(3)	4 980(5)	4 054(2)
C(231)	1 106(5)	5 424(9)	4 694(4)
C(232)	804(6)	6 976(8)	4 661(5)
O(231)	1 209(5)	4 621(7)	5 194(3)
O(24)	38(2)	2 849(5)	3 662(2)
C(241)	−430(4)	3 322(8)	3 017(4)
C(242)	−1 315(4)	3 922(9)	3 087(4)
O(241)	−134(3)	3 217(7)	2 486(3)

Table 2. Distances (Å) with e.s.d.s

C(1)–C(2)	1.449(7)	C(24)–C(25)	1.387(10)
O(1)–C(10)	1.383(7)	C(24)–O(24)	1.408(7)
C(2)–C(3)	1.531(8)	C(25)–C(26)	1.404(9)
C(2)–C(21)	1.515(8)	C(31)–C(32)	1.381(9)
C(3)–O(4)	1.454(6)	C(31)–C(36)	1.377(9)
C(3)–C(31)	1.511(8)	C(32)–C(33)	1.397(12)
O(4)–C(5)	1.384(7)	C(33)–C(34)	1.358(13)
C(5)–C(6)	1.388(8)	C(34)–C(35)	1.347(14)
C(5)–C(10)	1.372(8)	C(35)–C(36)	1.385(10)
C(6)–C(7)	1.371(9)	C(121)–C(122)	1.377(9)
C(7)–C(8)	1.390(8)	C(121)–C(126)	1.389(9)
C(8)–C(9)	1.387(8)	C(122)–C(123)	1.370(11)
C(8)–C(11)	1.477(8)	C(123)–C(124)	1.368(12)
C(9)–C(10)	1.393(8)	C(124)–C(125)	1.383(13)
C(11)–C(12)	1.336(9)	C(125)–C(126)	1.377(10)
C(12)–C(121)	1.486(8)	O(23)–C(231)	1.350(9)
C(21)–C(22)	1.395(9)	C(231)–C(232)	1.506(11)
C(21)–C(26)	1.348(9)	C(231)–O(231)	1.192(10)
C(22)–C(23)	1.396(8)	O(24)–C(241)	1.362(8)
C(23)–C(24)	1.340(9)	C(241)–C(242)	1.498(9)
C(23)–O(23)	1.419(8)	C(241)–O(241)	1.192(8)

in good yield by a BBr_3 (as a complex with dimethyl sulphide) demethylation of (*E*)-3-methoxystilben-4-ol.[14] The latter was synthesized (68% yield) *via* a Horner-Wittig reaction, the Wittig reaction gave only a 40% yield.

The (*E*)-stilbene-3,4-diol was mildly fungitoxic whilst the dihydrobenzofuran was inactive. The benzodioxin (**5a**) has been submitted for testing.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. I.r. spectra refer to KBr discs unless otherwise indicated. 300 MHz ^{1}H n.m.r. and ^{13}C n.m.r. respectively were recorded on a Brucker WH 300 spectrometer. Merck silica gel HF_{254} was used for t.l.c. [developer: chloroform–methanol (9.5:0.5)], spots were detected in u.v. light or in iodine vapour. Horseradish peroxidase P 8250, Sigma Chemical Co.

(E)-*Stilbene*-3,4-*diol* (**4**).—(*E*)-3-Methoxystilben-4-ol[12] (3 g) was added to a solution of BBr_3·SMe_2 (8.25 g) in 1,2-dichloroethane (300 ml) under nitrogen. The reaction mixture was refluxed for 6 h, poured into water, and extracted with ethyl acetate. The extract was washed ($NaHCO_3/H_2O$) and the solvent evaporated. The residue, which crystallised from acetone–benzene, afforded (*E*)-stilbene-3,4-diol (2.4 g) in platelets, m.p. 168—169 °C (Found: C, 79.05; H, 5.7. $C_{14}H_{12}O_2$ requires C, 79.22; H, 5.7%); $\nu_{max.}$(KBr) 3 336, 1 596, and 964 cm^{-1}; $\lambda_{max.}$(MeOH), 231 (ε 14 101), 292sh (16 651), 301 (18 095), and 322 (21 464) nm; δ_H [60 MHz; $(CD_3)_2CO$; standard Me_4Si], 3.16 (1 H, br s, OH, exchange D_2O), 7.0 (1 H, d, *J* 8.7 Hz, 5-H), 7.09 (1 H, d, *J* 2 Hz, 2-H), 7.23 (2 H, s, α- and β-H), 7.26—7.46 (1 H, m, 6-H), 7.46—7.86 (5 H, m, Ph), and 8.11 (1 H, br s, OH, exchanges D_2O); *m/z* 212 (M^+).

Enzymatic Oxidation of (E)-*Stilbene*-3,4-*diol with Peroxidase*.—A solution of compound (**4**) (1 g) and horseradish peroxidase (2 mg) in acetone (35 ml) and water (10 ml) was treated with hydrogen peroxide (3%; 5 ml) at 21 °C and stirred for 5 h. The acetone was removed and the aqueous reaction mixture extracted with ethyl acetate. The extract was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by p.t.l.c. (chloroform–methanol 9.5:0.5 as developer) to afford an oil (200 mg) which, on acetylation [acetic anhydride (2 ml)–pyridine (1.5 ml) at room temperature for 16 h followed by dilution], afforded a solid which was crystallised from acetone–benzene to give 3-(3,4-*diacetoxyphenyl*)-2,3-*dihydro*-2-*phenyl*-6-(2-*phenylvinyl*)-1,4-*benzodioxin* (**5b**) as plates (205 mg), m.p. 190—192 °C (Found: C, 75.5; H, 5.1. $C_{32}H_{26}O_6$ requires C, 75.87; H, 5.18%); $\nu_{max.}$(KBr) 1 778, 1 766, 1 591, and 971 cm^{-1}; $\lambda_{max.}$(MeOH) 235 (ε 19 836), 291sh (22 782), 302 (25 531), and 317 nm (28 084); δ_H [300 MHz; solvent $(CD_3)_2CO$] 2.27 (3 H, s, $COCH_3$), 2.28 (3 H, s, $COCH_3$), 4.88 (1 H, d, *J* 7.9 Hz, 3-H), 4.94 (1 H, d, *J* 8 Hz, 2-H), and 6.97—7.56 (15 H, m, Ar and olefinic H); for ^{13}C n.m.r. results ($CDCl_3$) see Figure 1.

The second band afforded an oil (105 mg). An aliquot (50 mg) was acetylated with acetic anhydride (0.5 ml) and pyridine (0.5 ml) at room temperature for 16 h, diluted with water and extracted with ethyl acetate. The extract was evaporated under reduced pressure and the oil obtained purified by p.t.l.c. (developer: chloroform) to give 2-(3,5-diacetoxyphenyl)-3-phenyl-5-(2-phenylvinyl)-1-benzofuran (**6b**), M^+ at *m/z* 548; $\nu_{max.}$($CHCl_3$) 1 765, 1 730, 1 595, and 950 cm^{-1}; $\lambda_{max.}$(MeOH) 218sh (ε 20 036), 236sh (12 360), 300sh (17 437), 308 (17 825), 320sh (15 873), and 335 nm (11 059); δ_H [270 MHz; $(CD_3)_2CO$] 2.04 (3 H, s, $COCH_3$), 2.42 (3 H, s, $COCH_3$), 2.46 (3 H, $COCH_3$), 4.86 (1 H, d, *J* 7.69 Hz, 3-H), 5.88 (1 H, d, *J* 7.3 Hz), and 8.47—8.98 (17 H, m, Ar and olefinic H).

Acknowledgements

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