Oxidative Dimerisation of 4-Hydroxystilbenes *in vitro*: Production of a Grapevine Phytoalexin Mimic

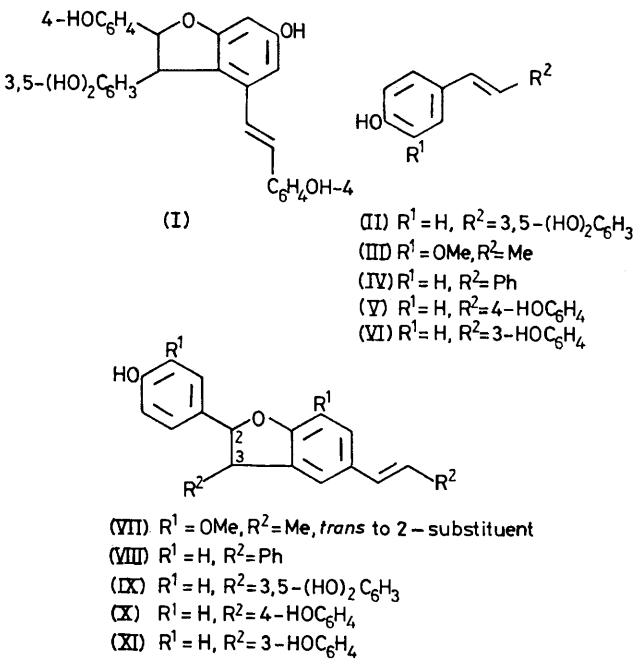
By PETER LANGCAKE and ROBERT J. PRYCE*

(*Shell Research Ltd., Shell Biosciences Laboratory, Sittingbourne Research Centre, Sittingbourne, Kent* ME9 8AG)

Summary Dimerisation of *trans*-4-hydroxystilbenes with horseradish peroxidase–hydrogen peroxide gives analogous products to those formed from phenylpropenoids; the product (IX) obtained from *trans*-resveratrol is structurally related to the phytoalexin (I) isolated from grapevines but the coupling orientation is different, and both the natural and synthetic dimers of resveratrol have a similar spectrum of antifungal activity.

WE have recently shown[1] that one of the antifungal compounds produced in grapevine (*Vitis vinifera*) leaves in response to fungal infection or u.v. irradiation is ε-viniferin (I), a putative dimer of *trans*-resveratrol (II). At the same time other phytoalexins, which appear to be related, higher oligomers of resveratrol, and the non-antifungal *trans*-resveratrol itself are also produced.[1] None of these phytoalexins nor *trans*-resveratrol can be detected in healthy vine leaves but mature vine wood contains some of them in considerable quantity.[1] It was proposed[1] that ε-viniferin could be biosynthesised by a controlled oxidative dimerisation of *trans*-resveratrol. Analogous reactions *in vitro* with 4-hydroxyphenylpropenoids have been carried out using both enzymes and chemical oxidants.[2] For example, oxidation of *trans*-isoeugenol (III) with horseradish peroxidase (HRP)–hydrogen peroxide gave the racemic dehydrodi-isoeugenol (VII) one optical isomer of which is now also known as a natural lignan, licarin A.[3]

In order to test our biosynthetic hypothesis and to see if the model phenylpropenoid lignan syntheses could be extended to stilbenes we chose to treat *trans*-4-hydroxystilbene (IV) with HRP–hydrogen peroxide under similar[2] conditions. A crystalline dehydrodimer was isolated (73% yield) m.p. 175—178 °C, $C_{28}H_{22}O_2$, M^+ at *m/e* 390, to which we assign structure (VIII) on the following evidence. By mass and ^{1}H-n.m.r. spectroscopy of the methylated (diazomethane) and acetylated derivatives the product was clearly a monophenol. Its u.v. spectrum showed a similar



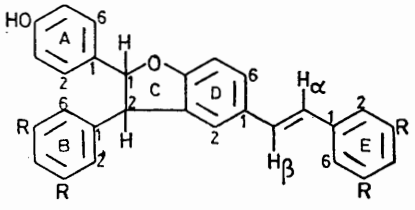
trans-stilbene chromophore to that of (IV), but in contrast to (IV) the long wavelength chromophore was unaffected by addition of aqueous sodium hydroxide, indicating the absence of a free *p*-hydroxy group on the *trans*-stilbene unit. Its i.r. spectrum indicated no other functionality. Assignments of the ^{1}H- and ^{13}C-n.m.r. spectra of (VIII) are given in the Table and Figure, respectively. Orientation of the A and B rings relies on the different line-widths of the ring C protons which are attributable to benzylic ^{1}H couplings between 2c-H and rings B and D compared with smaller

TABLE. 100 MHz ^{1}H-n.m.r. spectra of (VIII) and (IX) [$(CD_3)_2CO$, Me_4Si internal standard]. See structure (A) for assignments.

Compound	2C	1C	Proton assignments [δ p.p.m. (*J* Hz)$W_{\frac{1}{2}}$/Hz] 2B+6B	4B + 4E	2E+6E	3A+5A	2A+6A	5D	2D	6D	Hα	Hβ	OH
VIII, R = H)	4·62 d (8·5)$W_{\frac{1}{2}}$ (2·5)	5·49 d (8·5)$W_{\frac{1}{2}}$ (1·5)	← 6·76—7·54 complex m →										2·87 s
(IX, R = OH)	4·44 d (8·0)$W_{\frac{1}{2}}$ (3)[a]	5·46 d (8·0)$W_{\frac{1}{2}}$ (1·7)[a]	6·20 d (2·2)	6·28 m	6·55 d (2·0)	6·84 d (8·7)	7·22 d (8·7)	6·86 d (8·5)	7·26 brs [a]	7·40 dd (8·5 and 1·5)	6·88 d (16·5)	7·06 d (16·5)	8·02 brs (5xOH)

[a] Irradiation of proton 2C sharpens the aromatic proton resonances 2B, 6B, 4B, 5D, 6D, and, particularly 2D which resolves to a doublet, *J* 1·5 Hz. Irradiation of proton 1c sharpens only the ring A aromatic protons.

benzylic couplings of 1c-H with ring A. In agreement with this assignment there is a down-field shift (0·08 p.p.m.) of 1c-H in the acetate of (VII) ($CDCl_3$) compared with the free phenol (VIII) ($CDCl_3$) while 2c-H remains unaffected.

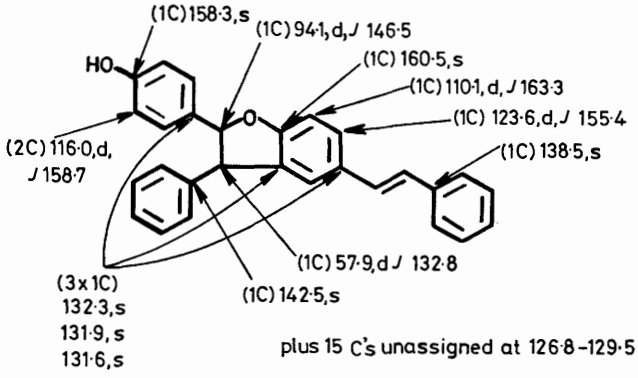


(A)

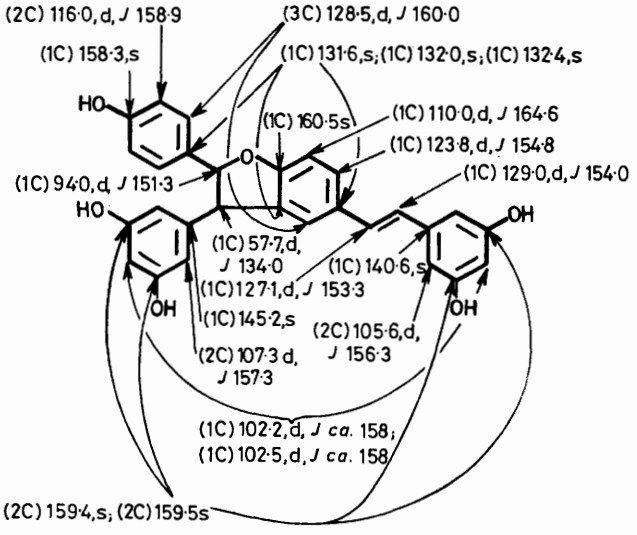
Assignment of the ^{13}C-n.m.r. spectrum of (VIII) was facilitated by prior analysis of the spectra of *trans*-4,4′-dihydroxystilbene (V) and *trans*-4-hydroxystilbene (IV).

When *trans*-resveratrol (II) was subjected to similar HRP–hydrogen peroxide treatment, ε-viniferin could not be detected but the major product was another isomeric dehydrodimer. It was isolated (41% yield) by high pressure liquid chromatography as a homogeneous (t.l.c. and g.l.c. on three different phases) amorphous solid and was shown to be a pentaphenol, $C_{28}H_{22}O_6$, by mass spectroscopy of the free phenol (M^+ at *m/e* 454), its pentamethyl ether (diazomethane) (M^+ at *m/e* 524·2188, $C_{33}H_{32}O_6$ requires M^+ 524·2199), and its penta-acetate (M^+ at *m/e* 664). ^{1}H-n.m.r. spectra of these derivatives confirmed the pentaphenolic nature of the product and structure (IX) is assigned to this dimer on the following evidence. Its u.v. spectrum had a similar *trans*-stilbene chromophore to that of *trans*-resveratrol[1] but in contrast it showed no base shift of the long wavelength chromophore, indicating the presence of a *trans*-stilbene unit lacking a free *p*-hydroxy group. Its i.r. spectrum indicated no additional functionality. ^{1}H- and ^{13}C-n.m.r. spectral assignments to (IX) are shown in the Table and Figure, respectively and ^{1}H-benzylic couplings were again used to determine the orientation of rings A and B. ^{13}C Assignments were facilitated by comparison with the spectra[1] of *trans*-resveratrol (II) and ε-viniferin (I). This new synthetic dehydrodimer of *trans*-resveratrol differs from ε-viniferin (I) in that only the presumed radical intermediates (*cf.* ref. 2) derived from the two *p*-hydroxy substituted aromatic rings have coupled to form (IX); formation of ε-viniferin would require coupling of radicals derived from one of each of the *p*-hydroxy and di-*m*-hydroxy rings.

The two stilbene dimers described here, (VIII) and (IX), are both racemic compounds; ε-viniferin is a single optical isomer.[1] No attempt has been made to use ^{1}H n.m.r. vicinal coupling constants to define the 2,3-stereochemistry of (VIII) and (IX) since such assignments in phenylpropenoid dimers of the type (VII) are known to be unreliable.[4] In the absence of any other information at present, analogy with the formation of (VII) from (III) suggests *trans*-2,3-stereochemistry for (VIII) and (IX).



(VIII)



(IX)

FIGURE. 25 MHz ^{13}C-n.m.r. spectra of (VIII) and (IX) [$(CD_3)_2CO$, Me_4Si internal standard]. Assignments: (number carbons with this chemical shift) δ p.p.m., multiplicity of signal in coupled spectrum, J_{CH} Hz.

Despite the different couplings of *trans*-resveratrol units in the synthetic dimer (IX) and ε-viniferin (I) sufficient structural similarity remains for the synthetic dimer to

mimic the antifungal properties of ϵ-viniferin. Both compounds have a similar spectrum and magnitude of activity *in vitro*[1] and *in vivo* against phytopathogenic fungi. While *trans*-4-hydroxystilbene shows antifungal properties in our bioassays[5] its dimer (VIII) is inactive. Oxidative dimerisation as described above, of *trans*-4,4′-dihydroxystilbene (V) and *trans*-3,4′-dihydroxystilbene (VI) has given the new antifungal dimers (X) (7% yield) and (XI) (46% yield), respectively.

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