Phytochemistry, 1977, Vol. 16, pp. 1452–1454. Pergamon Press. Printed in England.

α-VINIFERIN: AN ANTIFUNGAL RESVERATROL TRIMER FROM GRAPEVINES

R. J. PRYCE and P. LANGCAKE

Shell Research Ltd., Shell Biosciences Laboratory, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG, England

(*Received* 22 *March* 1977)

Key Word Index—*Vitis vinifera*; Vitaceae; α-viniferin; cyclic trimer; *trans*-resveratrol; stilbene oligomers

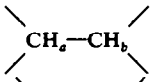
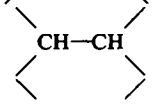
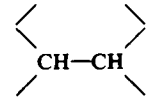
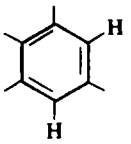
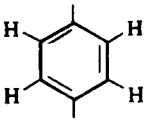
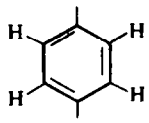
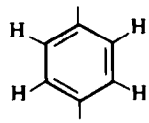
A new class of phytoalexins, the viniferins, has recently been isolated from the grapevine, *Vitis vinifera*. These phytoalexins appear to be oligomers of *trans*-resveratrol (1) and evidence for the structure of the dimer, ε-viniferin (2) has been presented [1]. We present here our evidence for the proposed structure (3) of the trimer, α-viniferin.

α-Viniferin was isolated from the weakly acidic fraction of UV-irradiated and *Botrytis cinerea*-infected, detached vine leaves by a combination of Sephadex LH-20 column chromatography (90% EtOH) and preparative TLC (Si gel; CH_2Cl_2–MeOH, 4:1) as a homogeneous (TLC as above and HPLC [2]) amorphous solid which gave a strong colour reaction with diazotised *p*-nitroaniline.

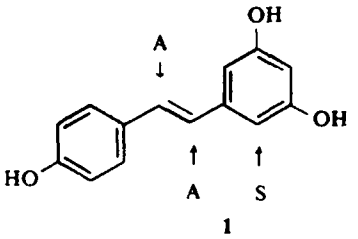
Electron impact MS of α-viniferin and its methyl (CH_2N_2) and acetyl (Ac_2O–Py room temp.) derivatives gave no satisfactory spectra. By field desorption MS these three compounds showed strong parent ions at *m/e* 678.1886 ($C_{42}H_{30}O_9$ requires 678.1889), 762 and 930 respectively. These data suggest that α-viniferin is a hexaphenol of molecular formula $C_{42}H_{30}O_9$. α-Viniferin has a UV spectrum {λ_{max}^{EtOH} nm (log ε): *sh*225 (4.68), *sh*281 (3.92), 286 (4.00), *sh*293 (3.93)} attributed to unconjugated phenolic chromophores and this assignment is in accord with its IR spectrum (γ_{max}^{KBr} cm^{-1}: 3440, 1613, *sh*1597, 1510, 831, 805, 775, 767).

PMR spectra of α-viniferin and its methyl and acetyl derivatives together with assignments based on chemical shifts and double resonance experiments are shown in Table 1. It has not been possible to obtain satisfactory ^{13}C NMR spectra with the quantities of material available. From the PMR spectra it is clear that α-viniferin is indeed a hexaphenol forming hexamethyl and hexaacetyl derivatives and therefore its formula, $C_{42}H_{30}O_9$, suggested by field desorption MS measurements is established. The assignments made to the PMR spectrum of α-viniferin and its derivatives indicate that it consists of three 1,4-disubstituted benzene rings, three 1,2,3,5-tetrasubstituted benzene rings and three >CH—CH< units. This is the composition that would be expected for a trimer of a phenolic stilbene such as the naturally occurring *trans*-resveratrol (1) if the trimerisation occurred by substitutions (S) and additions (A) as shown in (1). Chemical shifts of the three >CH—CH< units require that they should be α to deshielding groups such as phenyl and oxygen and this situation is possible if α-viniferin is derived by the suggested stilbene trimerisation. The putative monomer, *trans*-resveratrol (1) has also been isolated from *B. cinerea*-infected and UV-irradiated vine leaves and from vine wood [1, 3]. Taking the resveratrol trimerisation hypothesis further, α-viniferin ($C_{42}H_{30}O_9$) should be formed oxidatively from resveratrol ($C_{14}H_{12}O_3$). In addition to the six aromatic rings and six phenol groups in α-viniferin (Table 1, PMR) its formula requires that there should be four other rings and three ether oxygens since no other unsaturation or oxygen functions are indicated by its spectral properties. On the basis of all these data structure (3) is proposed for α-viniferin. This structure is related to that of a recently isolated *trans*-resveratrol dimer, ε-viniferin (2), from vine wood and UV-irradiated or *Botrytis cinerea*-infected vine leaves; in the latter it co-occurs with α-viniferin [1]. The proposed structure of α-viniferin (3) comprises three *trans*-2-aryl-2,3-dihydrobenzofuran units arranged in a *trans, cisoid, trans, transoid, trans* fashion to form a nine-membered ring. It is not at all certain that the 2,3-dihydrobenzofurans are all *trans* from the PMR coupling constants since 2,3-vicinal proton coupling constants in 2,3-dihydrobenzofurans have been found to be unpredictable [4, 5]. However, only *trans*-2-aryl-2,3-dihydrobenzofurans appear to have been found so far in plants [4–7]. For the present, and assuming all the dihydrobenzofuran units to be *trans*, then one of the units in α-viniferin is best put *transoid* to the other two for the following reasons: (a) α-viniferin has no three-fold axis of symmetry since it is optically active showing a complex circular dichroism spectrum {$\Delta\varepsilon_{300}$ + 7.4, $\Delta\varepsilon_{292}$ + 3.2, $\Delta\varepsilon_{275}$ + 0.9, $\Delta\varepsilon_{249}$ + 13.4, $\Delta\varepsilon_{229}$ − 11.7, $\Delta\varepsilon_{207}$ − 56.0 (EtOH; *c* 0.01270 and *c* 0.00127 for the last two minima with N_2 flushing} and the PMR spectra of α-viniferin and its derivatives (Table 1) show no degenerate resonances. Therefore, structure (3) with a *trans, cisoid, trans, cisoid, trans* arrangement which would have a three-fold axis of symmetry can be excluded. Structure (3) as drawn is asymmetric. (*b*) In a Dreiding model of (3) there is a torsion angle of *ca* 90° between hydrogens *a* and *b* of the *transoid-trans* dihydrobenzofuran moiety and, by consideration of the Karplus equation, this could explain the near zero coupling observed in the PMR spectra of α-viniferin and its derivatives (Table 1) for a pair of hydrogens assigned to a >CH—CH< group. (c) The *trans, cisoid, trans, transoid, trans*, structure of α-viniferin (3) with its inherent assymetry may be the reason for the somewhat anomalous proton chemical shifts which could be due to shielding and deshielding arising from the aromatic rings. In particular proton *Ha* (3 and Table 1) is

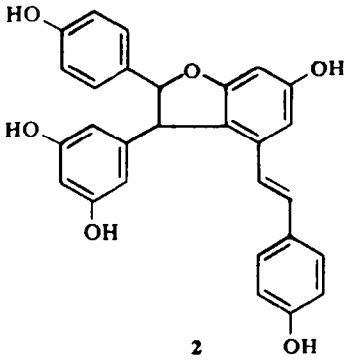
Table 1. 100 MHz PMR spectra and assignments of α-viniferin and its derivatives, δ ppm from TMS internal standard for solutions as described

No of protons	Assignments*	α-Viniferin (D_6-acetone)	α-Viniferin methyl ether (D_6-acetone)	α-Viniferin acetate ($CDCl_3$)
		δ, J or $W_{\frac{1}{2}}$(Hz)	δ, J or $W_{\frac{1}{2}}$(Hz)	δ, J or $W_{\frac{1}{2}}$(Hz)
1H 1H		(a) 3.98*s*, $W_{\frac{1}{2}}$3 (b) 6.06*s*, $W_{\frac{1}{2}}$3	(a) 3.98*s*, $W_{\frac{1}{2}}$3 (b) 6.21*s*, $W_{\frac{1}{2}}$3	(a) 3.99*s*, $W_{\frac{1}{2}}$3 (b) 6.12*s*, $W_{\frac{1}{2}}$3
1H 1H		4.62*d*, J6.0 4.92*d*, J6.0	4.70*d*, J6.0 4.93*d*, J6.0	4.68*d*, J6.0 4.86*d*, J6.0
1H 1H		4.72*d*, J10.0 5.95*d*, J10.0	4.75*d*, J10.0 6.11*d*, J10.0	4.73*d*, J11.0 6.07*d*, J11.0
1H 3H 1H 1H	3x 	5.99*d*, J2.0 6.23*m* 6.59*d*, J2.0 *ca* 6.7*d*, J *ca* 2	6.01*d*, J2.0 1H 6.33*d*, J2.0 2H 6.38*t*, J *ca* 2 *ca* 6.8*d*, J *ca* 2 6.75*d*, J2.0	6.22*d*, J2.0 6.60*m* 6.87*m*
2H 2H		‡ 6.71*d*, J9.0 7.02*d*, J9.0	6.85*d*, J9.0 7.08*d*, J9.0	complex unresolved 6.93 to 7.11
2H 2H		‡ 6.76*d*, J8.5 7.20*d*, J8.5	6.80*d*, J8.5 7.29*d*, J8.5	7.03*d*, J8.5 7.32*d*, J8.5
2H 2H		‡ 6.78*d*, J9.0 7.04*d*, J9.0	6.88*d*, J9.0 7.11*d*, J9.0	complex unresolved 6.93 to 7.11
OH's§ plus water in solvent	OH's	3.68 *brs*	—	—
18H	6 × $OC\underline{H}_3$	—	3.64*s* 3.75*s* 3.77*s* 3.80*s* 3.81*s* 3.82*s*	—
18H	6 × $OCO\underline{H}_3$	—	—	2.25*s* 2.28*s* 2.30*s* 2.31*s* 2.32*s* 2.33*s*

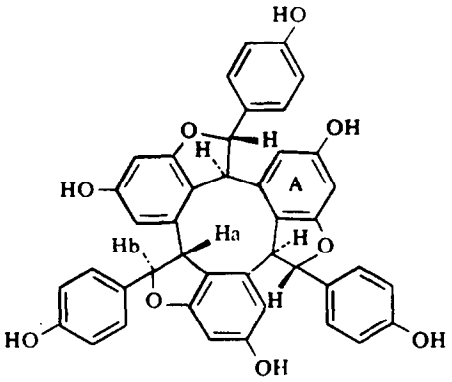
* Assignments based on double resonance experiments and chemical shifts.
† Indicates coupling between signals determined by double resonance experiments.
‡ These indicated 'doublets' were superficially doublets but on closer examination they are more complex AA'BB'systems—the observed J_{AB}'s are quoted.
§ By D_2O exchange.



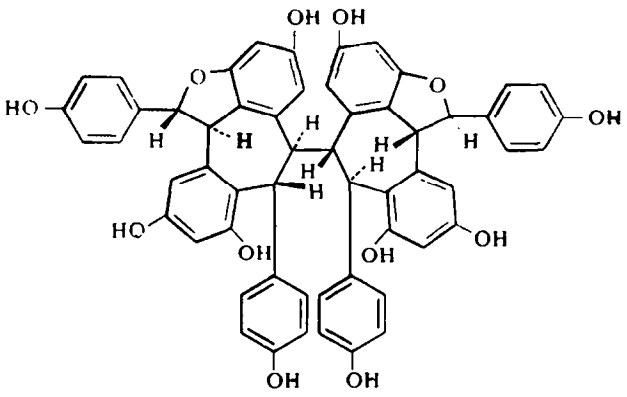
1



2



3



4

seen to resonate at rather high field and in the Dreiding model it resides directly above the plane of aromatic ring A; this situation could account for the observed diamagnetic shift.

Prior to our isolation of the viniferins only one stilbene oligomer, hopeaphenol (4) was known as a constituent of the wood of some species of Dipterocarpaceae [7]. Hopeaphenol bears some structural similarities to the proposed structure of α-viniferin (3); in particular it has *trans*-2-aryl-2,3-dihydrobenzofuran moieties. Some of the antifungal properties of α-viniferin have been reported previously [1].

REFERENCES

1. Langcake, P. and Pryce, R. J. (1977) *Experientia* **33**, 151.
2. Langcake, P. and Pryce, R. J. (1977) *Phytochemistry* **16**, 1193.
3. Langcake, P. and Pryce, R. J. (1976) *Physiol. Plant Path.* **9**, 77.
4. Gregson, M., Ollis, W. D., Redman, B. T., Sutherland, I. O. and Dietrichs, H. H. (1968) *J. Chem. Soc. Chem. Commun.* 1394 and references therein.
5. Donnelly, B. J., Donnelly, D. M. X., O'Sullivan, A. M. and Prendergast, J. P. (1969) *Tetrahedron* **25**, 4409.
6. Aiba, C. J., Campos Correa, R. G. and Gottlieb, O. R. (1973) *Phytochemistry* **12**, 1163.
7. Madhav, R., Seshadri, T. R. and Subramanian, G. B. V. (1967) *Phytochemistry* **6**, 1155 and references therein.