SPECIALIA

A new class of phytoalexins from grapevines

P. Langcake and R. J. Pryce

Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent, ME9 8AG (England), 13 July 1976

Summary. Novel phytoalexins which have been isolated from grapevine leaves are oligomeric forms of the trihydroxy stilbene resveratrol, which co-occurs with these compounds. The characterization of one of these phytoalexins as a resveratrol dimer, designated ε-viniferin, is described as well as a model for its biosynthesis from resveratrol.

The production of phytoalexins, antimicrobial compounds produced by plants in response to infection, is widely believed to be an important disease resistance mechanism[1]. Although many phytoalexins have now been characterized (e.g.[2]), the majority of these are produced by members of the Leguminosae and the Solanaceae. Insufficient studies of other plant groups have been made to support the suggestion that phytoalexin production is a universal disease resistance mechanism in higher plants.

We have discovered a new class of phytoalexin from grapevine (Vitis vinifera L.) for which we propose the trivial generic name viniferins, and whose production may be a common feature of the vine family (Vitaceae). Extraction of detached vine leaves which had been infected with Botrytis cinerea or irradiated with UV light and subsequent bioassay of the extracts on silica gel thin-layer chromatograms using Cladosporium cucumerinum[3] has revealed the presence of several weakly acidic antifungal components. Inhibitory compounds cannot be detected by this technique in comparable extracts of healthy leaves.

One of these antifungal compounds, designated ε-viniferin, was isolated as a chromatographically homogeneous amorphous solid m.p. 155–160 °C, using Sephadex LH-20 and silica gel column chromatography. From its colour reaction with diazotised p-nitroaniline and mass spectrometry of ε-viniferin (P^+ 454.1410), its pentamethyl (P^+ 524) (diazomethane/ether in methanol) and penta acetyl (P^+ 664) (acetic anhydride/pyridine) derivatives, ε-viniferin was clearly a pentaphenol of formula $C_{28}H_{22}O_6$ (M^+ requires m/e 454.1416). Its UV spectrum [λ_{max}^{EtOH} nm (ε), 224 (54200), sh 286 (15100), 310 (22800), 324 (24300), sh 345 (132000)] suggested that ε-viniferin might contain a trans stilbene moiety. Trans-resveratrol (I, $C_{14}H_{12}O_3$) has a similar long wavelength chromophore [λ_{max}^{EtOH} nm (ε), 218 (19100), sh 235 (11800), sh 297 (24400), 306 (26800), 320 (25900), sh 336 (16700)] and this chromophore of both ε-viniferin and trans-resveratrol shifted to higher wavelengths on the addition of 1 N sodium hydroxide, indicating the presence of a phenolic hydroxyl group in the 2- or 4-position of the stilbene moiety. Detailed analysis of the 100 MHz ^{1}H-NMR-spectra of ε-viniferin [(δ ppm from TMS for D_6-acetone solution, multiplicity of signal, coupling constant and integral) 4.50, d, J 5.4 Hz (1H): 5.45, d J 5.4 Hz (1H): 6.27, s (3H): 6.35, d, J 2.1 Hz (1H): 6.75, d, J 2.1 Hz (1H): 6.76, d, J 8.5 Hz (2H): 6.86, d, J 8.5 Hz (2H): 7.17, d, J 8.5 Hz (2H): 7.22, d, J 8.5 Hz (2H): 6.73, d, J 16.0 Hz (1H): 6.93, d, J 16.0 Hz (1H): 8.40, brs (5 × OH)] and its methyl and acetyl derivatives and the ^{13}C-NMR-spectrum of ε-viniferin (figure) indicated that this compound has the structure **II**. ε-Viniferin is thus a dehydro dimer of resveratrol (**I**). The assignments of the ^{13}C-NMR-spectrum of trans-resveratrol (**I**) are shown for comparison

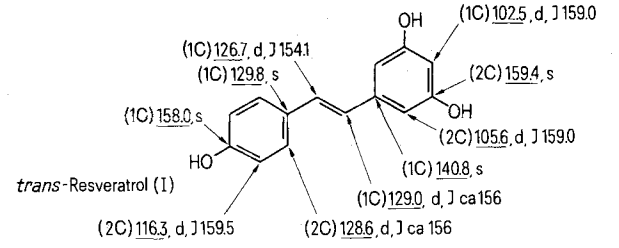
1 J. Kuć, Ann. Rev. Phytopath. *10*, 207–232 (1972).
2 J. L. Ingham, Phytopathol. Z. *78*, 314–335 (1973).
3 J. A. Bailey and R. S. Burden, Phys. Plant Path. *3*, 171–177 (1973).

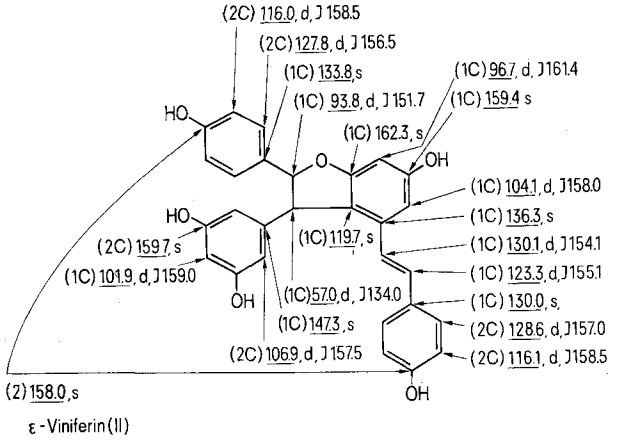
Antifungal activity of resveratrol and derivatives

	Tlc plate assay*	Spore germination tests**			Plasmopara viticola***	
		Botrytis cinerea	Cladosporium cucumerinum	Piricularia oryzae	Zoospore release	Zoospore motity
Resveratrol	>60	>200	>200	>200	>200	>200
α-viniferin	1	97	47	28	35	11
γ-viniferin	9	>200	150	57	>100	>100
ε-viniferin	2.5	100	37	n.t.	19	12.5
Compound **VI**	2	125	30	30	16	5

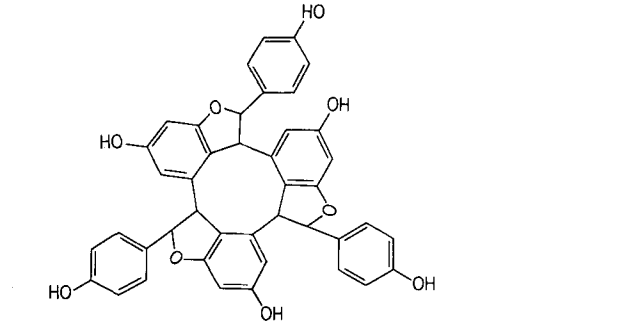
*Minimum amount detectable (μg) by Cladosporium cucumerinum tlc plate assay[3]. **Concentrations (μg/ml) causing 50% inhibition of spore germination. ***Concentrations (μg/ml) causing 50% inhibition of release of zoospores from sporangia of Plasmopara viticola, or the motility of the zoospores after their release[6]. Bioassays were carried out in duplicate and the results represent the mean values of at least 2 independent determinations.

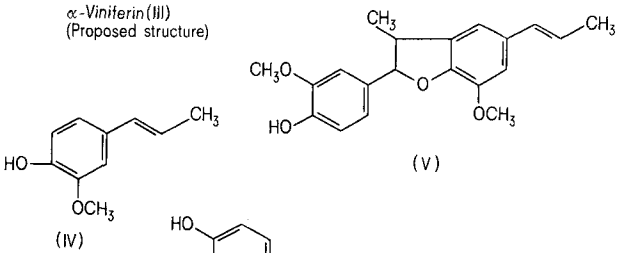
(figure). The anticipated[4] and rapid light-induced isomerisation of the stilbene moiety in ε-viniferin (**II**) to the cis-isomer [λ_{max}^{EtOH} nm (ε), sh 262 (14300), 274 (18700), 283 (17700), sh 294 (14300), sh 319 (7850)] was observed during isolation of ε-viniferin from leaves when extracts

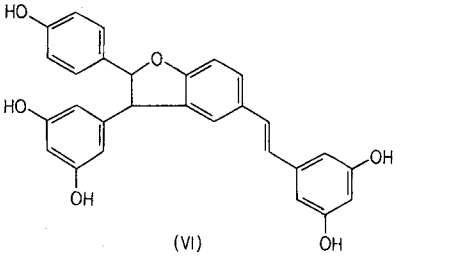




25 MHz ^{13}C-NMR-spectra and assignments of ε-viniferin and trans-resveratrol for D_6-acetone solutions. Assignments (No. of carbons with this chemical shift, etc.): δ ppm from TMS internal standard, multiplicity of signal in undecoupled spectrum, $J_{C,H}$ Hz.







were not protected from daylight. Cis-ε-viniferin had a practically identical mass spectrum to trans-ε-viniferin but a distinct ^{1}H-NMR-spectrum.

The proposed structure (**II**) suggested that trans-resveratrol (**I**) and other oligomers of resveratrol might co-occur with ε-viniferin in both UV-irradiated and infected leaves and this was found to be the case. We have recently described[6] the production of trans-resveratrol in grapevine leaves as a response to infection or UV irradiation. In addition we have isolated three other components from leaves irradiated with UV light or infected with B. cinerea. These are designated α-viniferin (proposed structure **III**) – a cyclic trimer of resveratrol, β-viniferin which is probably a cyclic tetramer of resveratrol (although different from the only previously described resveratrol oligomer, hopeaphenol[5]) and γ-viniferin which is believed to be a more highly polymerised oligomer of resveratrol. α-Viniferin (**III**) was the major antifungal compound detected in leaves infected with B. cinerea (concentration >50 μg/g fresh weight) while the concentration of ε-viniferin was estimated to be 10 μg/g. In addition to α-viniferin (20 μg/g) and ε-viniferin (15 μg/g), UV irradiated leaves contained β-viniferin (9 μg/g) and γ-viniferin (80 μg/g), together with other unidentified antifungal components. Resveratrol, although not a normal constituent of the leaves, was present as a major component (ca. 700 μg/g) of apparently normal lignified stem tissues of grapevine[6]. ε-Viniferin was also isolated from the same source (ca. 500 μg/g) which contains several other antifungal components which react with diazotised p-nitroaniline.

The antifungal activities of resveratrol, α, γ and ε-viniferins have been compared (table). Although resveratrol has slight activity against fungal mycelial growth on an agar medium[6], the generally low activity of this compound suggests that, unlike the viniferins, resveratrol should not be considered a phytoalexin. Resveratrol probably functions as the biosynthetic precursor of the viniferins. We propose that the viniferins are biosynthesized from resveratrol by an oxidative process analogous to that involved in the formation of the dimeric phenyl propenoid lignans, e.g. the dehydro dimer of isoeugenol (**IV**), named licarin A (**V**)[7,8]. Support for this hypothesis was obtained when resveratrol was treated with horseradish peroxidase and H_2O_2. The major product (40% yield) of this reaction was the antifungal (table) resveratrol dimer **VI**, of structure analogous to that of ε-viniferin. Coupling of this compound, or ε-viniferin, with additional units of resveratrol could occur by the same process. Similar coupling reactions have been achieved with other 4-hydroxylated stilbenes.

Previous workers[9] have noted the chemotaxonomic significance of the majority of phytoalexins isolated so far. Our finding that resveratrol production is a response of all members of the Vitaceae examined[6] and that each of these plants produced compounds with similar chromatographic properties to the viniferins suggests that the production of viniferin-like compounds may be a characteristic of the family Vitaceae. Full experimental details of the work reported here will be published elsewhere.

4 E. V. Blackburn and C. J. Timmons, Q. Rev. *23*, 482–503 (1969).
5 P. Coggan, T. J. King and S. C. Wallwork, Chem. Comm. 439 (1966).
6 P. Langcake and R. J. Pryce, Phys. Plant Path. *9*, 77 (1976).
7 C. J. Aiba, R. G. Correa and O. R. Gottlieb, Phytochemistry *12*, 1163 (1973).
8 K. V. Sarkanen and A. F. A. Wallis, J. Chem. Soc. Perkin *I*, 1869 (1973).
9 J. L. Ingham and J. B. Harborne, Nature *260*, 241 (1976).