Polyphenols from Dipterocarp Species. Vaticaffinol and ε-Viniferin

Subramaniam Sotheeswaran,* M. Uvais S. Sultanbawa, and Sivagnanasundram Surendrakumar
Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka
Sinnathamby Balasubramaniam
Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka
Peter Bladon
Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, U.K.

A dipterocarp endemic to Sri Lanka, *Vatica affinis*, yielded three phenols. Two of them showed antibacterial properties and have been shown to be the dimer and tetramer of 3,5,4'-trihydroxystilbene.

Vatica affinis Thw. is a dipterocarp species endemic to Sri Lanka. The light petroleum extractives of the bark and timber of this species have been chemically investigated[1] and the following have been isolated: β-amyrin acetate, β-amyrin, β-sitosterol, hexamethoxycoeruleoellagic acid, tetramethoxyellagic acid, ursolic acetate, scopoletin, and betulinic acid. The cold acetone extract of *V. affinis* has now been investigated and here we report the characterisation of the polyphenols from the bark extractives.

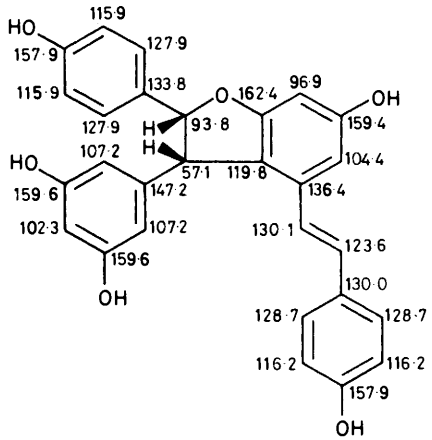
Results and Discussion

The acetone extract was separated on a silica-gel column. Elution with acetone–benzene (3:7) gave the first polyphenol as an off-white solid, m.p. 146—148 °C, molecular formula $C_{28}H_{22}O_6$ (M^+, 454.1415). Methylation with dimethyl sulphate gave a pentamethyl ether and acetylation with acetic anhydride–pyridine gave a penta-acetate. U.v. data indicated the presence of a *trans*-stilbene moiety. Although a bathochromic shift was observed when sodium hydroxide was added, no shift was observed with added sodium acetate–boric acid. The i.r. spectrum of the polyphenol had strong absorptions at 3 230 (OH) and 1 595 cm^{-1} (aromatic C=C). The pentamethyl ether, however, had no OH absorptions in its i.r. spectrum. These data suggested that the non-hydroxylic oxygen atom of the polyphenol occurs as an ether linkage. The molecular formula is in agreement with a dimeric form of 3,5,4'-trihydroxystilbene (resveratrol).

The ^{1}H n.m.r. spectrum showed two doublets at δ_H 4.45 and 5.42 (1 H each, J 6 Hz) and overlapping multiplets in the aromatic region integrating for 13 H. The coupled protons at δ_H 6.31 (1 H, d, J 2 Hz) and at δ_H 7.30 (1 H, d, J 2 Hz) are due to olefinic protons. The off-resonance ^{13}C n.m.r. spectrum was very informative. It had four singlets (δ_C 157—163 p.p.m.) for six phenolic carbon atoms, eight doublets (96—129 p.p.m.) for aromatic carbon atoms, two doublets (123.6 and 130.1 p.p.m.) for olefinic carbon atoms, and two further doublets (57.1 and 93.8 p.p.m.) for aliphatic carbon atoms.

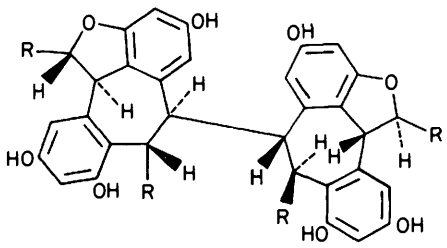
These data are in agreement with structure (**1**) for the polyphenol. The polyphenol is thus identical with *trans*-ε-viniferin (a resveratrol dimer) reported previously[2] from infected grape vine leaves (*Vitis vinifera*). The ^{13}C n.m.r. chemical shift assignments are given in Figure 1.

The silica-gel column on elution with acetone–benzene (7:13) gave a further polyphenol named vaticaffinol[3] (**5**), m.p. 280 °C (decomp.), $[\alpha]_D$ −22.5° (MeOH). Vaticaffinol gave a decamethyl ether, m.p. 160—162 °C, $[\alpha]_D$ +20.9° ($CHCl_3$), M^+, 1 046.4253 ($C_{66}H_{62}O_{12}$), with dimethyl sulphate and a deca-acetate m.p. 154—156 °C, $[\alpha]_D$ −33.9° ($CHCl_3$) with acetic anhydride in pyridine at room temperature. This indicated that 10 of the 12 oxygen atoms are present as hydroxy groups. Two of the remaining oxygen atoms should be present as ether linkages since the i.r. spectrum of the decamethyl ether did not show any absorptions for OH or CO. The u.v. spectrum showed absorption maxima at $\lambda_{max.}$ 288 (log ε 4.44) similar to those obtained for other polyphenols from dipterocarp species.[4] No shifts were observed with added sodium acetate–boric acid. Concentrated nitric acid oxidation of vaticaffinol (**5**) at 100 °C yielded picric acid, and 3-nitroanisic acid was obtained from a similar oxidation of decamethylvaticaffinol. The alkali fusion of vaticaffinol at 270 °C gave *p*-hydroxybenzoic acid. These data, the isolation of a resveratrol dimer, ε-viniferin, from the same extract and the molecular formula suggested that vaticaffinol is a resveratrol tetramer. The resveratrol tetramer, hopeaphenol[5] (**2**) was the first of this type of polyphenol. The physical properties of hopeaphenol (**2**) and its derivatives were found to be different (see Table) from those of vaticaffinol (**5**) and its derivatives. The ^{1}H n.m.r. spectrum of hopeaphenol decamethyl ether showed 4 aliphatic proton signals and 5 OCH_3 signals, showing a C_2-axis of symmetry, whereas the ^{1}H n.m.r. spectrum of vaticaffinol decamethyl ether showed eight aliphatic proton signals and 10 OCH_3 signals. The presence of a strong $M^+/2$ fragment ion in the mass spectrum of hopeaphenol[5] (**2**) is in agreement with its symmetric structure. The absence of such a strong $M^+/2$ fragment ion for vaticaffinol (**5**) and the above data point to a disymmetric configuration for vaticaffinol (**5**), different from hopeaphenol (**2**). The isolation of the resveratrol dimer, *trans*-ε-viniferin (**1**) ($C_{28}H_{22}O_6$) from the same extractives suggests

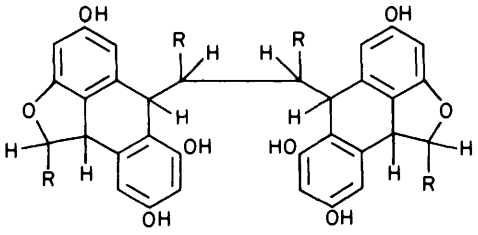


(1)

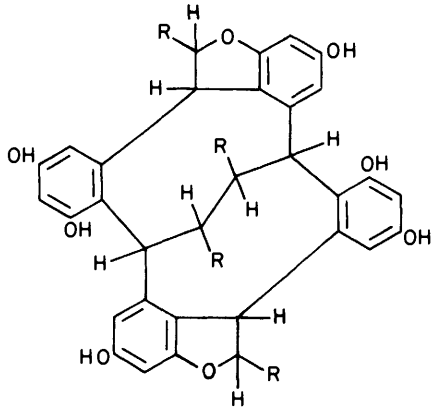
Figure 1. ^{13}C N.m.r. chemical-shift assignments of ε-viniferin



(2) R = C_6H_4OH-*p*



(3) R = C_6H_4OH-*p*



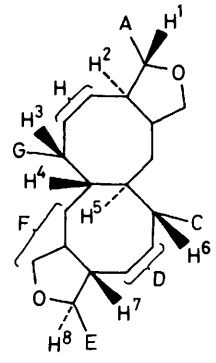
(4) R = C_6H_4OH-*p*

Table. Physical properties of compounds (**2**) and (**5**) and some derivatives

	M.p. (°C)	$[\alpha]_D$ (°)
Vaticaffinol (**5**)	280	−22.5
Hopeaphenol (**2**)	350	−407
Vaticaffinol deca-*O*-methyl ether	160—162	+20.9
Hopeaphenol deca-*O*-methyl ether	162—164	−378
Vaticaffinol deca-acetate	154—156	−33.9
Hopeaphenol deca-acetate	249—250	−338

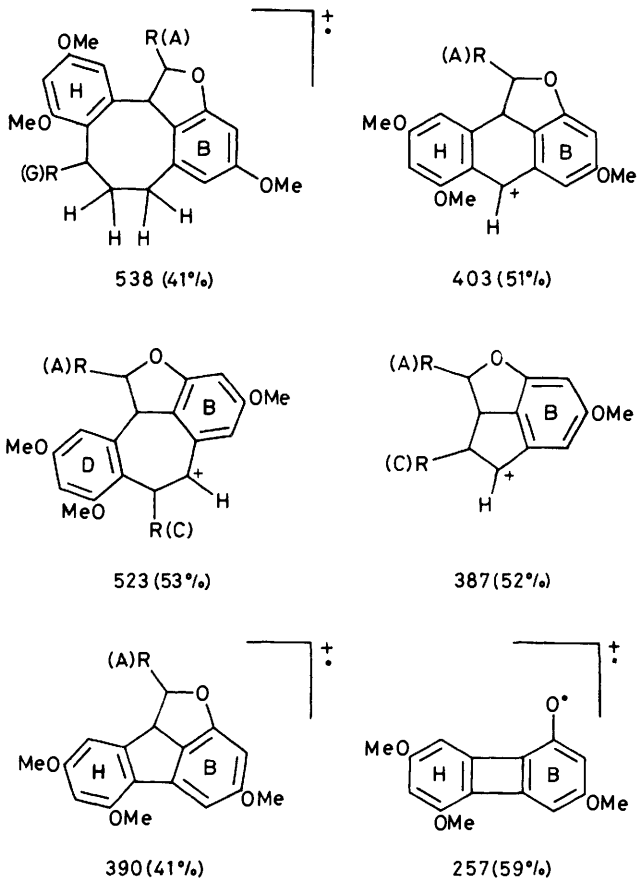
that vaticaffinol (**5**) ($C_{56}H_{42}O_{12}$) is possibly a resveratrol tetramer and that it could have been formed by biosynthetic dimerisation of *trans*-ε-viniferin (**1**). Four different structures (**2**)—(**5**) would arise by a different phenolic coupling mechanism; one is hopeaphenol (**2**). Construction of the models shows that (**3**) is non-rigid and cannot be a stable entity. Though (**4**) explains most of the observed data, stereochemically it is the least probable one and does not explain the chemical shifts and the observed coupling constants; (**5**) explains the observed spectroscopic and chemical data for vaticaffinol.

The off-resonance ^{13}C n.m.r. spectrum of vaticaffinol in [$^2H_6$]acetone showed 8 doublets (δ_C 37 to 95 p.p.m.) for aliphatic carbons, 10 singlets (δ_C 155 to 163) for 12 phenolic carbons and 13 doublets (96 to 131 p.p.m.) for 24 aromatic carbon atoms. The ^1H n.m.r. spectrum at high frequency (270, 360 MHz) showed resonances for 24 aromatic protons and 8 coupled methine protons in the region δ_H 3.1 to 5.8. Decoupling studies (see Experimental section for details) on the methine protons showed that 4 adjacent protons were coupled to each other. Two other pairs were also found to be mutually coupled to each other. The assignments [see (A)] are: δ_H 5.76 (d, 1-H),



(A)

4.44 (d, 2-H), 5.21 (d, 3-H), 3.19 (4-H), 4.09 (dd, 5-H), 4.55 (d, 6-H), 4.68 (d, 7-H), and 5.37 (d, 8-H); $J_{1,2}$ 11.5, $J_{3,4}$ 3.66, $J_{4,5}$ 11.0, $J_{5,6}$ 10.0, and $J_{7,8}$ 5.15 Hz. These and the structures of the mass fragments (Figure 2) are in keeping with structure (**5**) proposed for vaticaffinol. Complete ^{13}C n.m.r. chemical shifts for vaticaffinol are given in Figure 3.

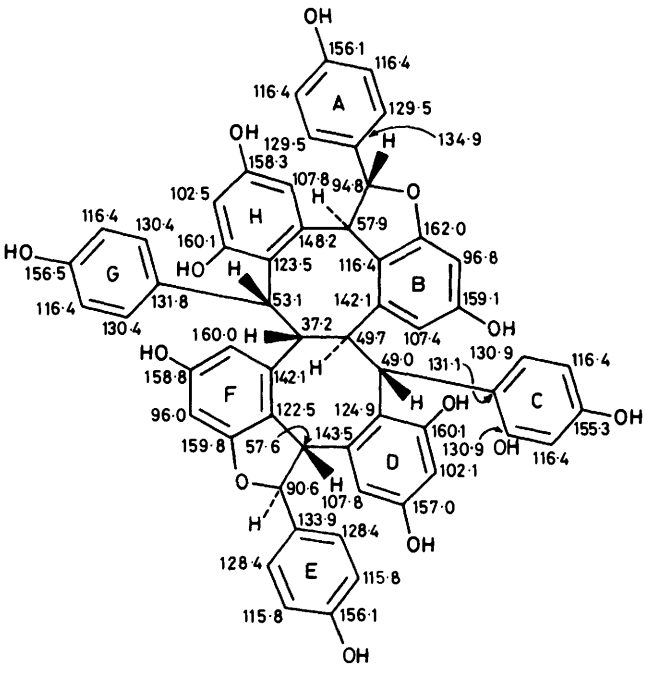


538 (41%) 403 (51%)

523 (53%) 387 (52%)

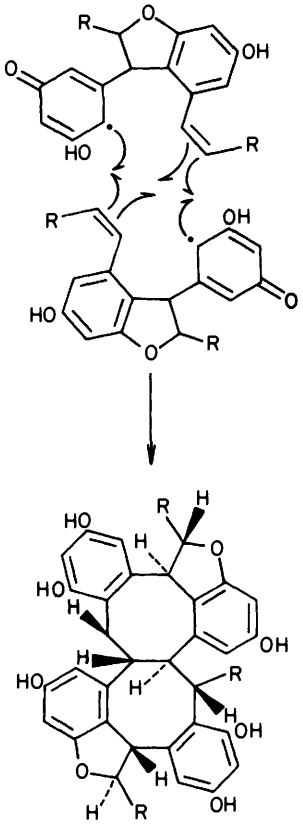
390 (41%) 257 (59%)

Figure 2. Mass spectral fragmentations of deca-*O*-methylvaticaffinol (R = C_6H_4OMe-*p*)



(5)

Figure 3. ^{13}C N.m.r. chemical-shift assignments of vaticaffinol (**5**)

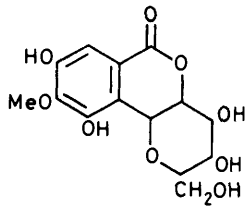


Scheme. Biosynthetic formulation of vaticaffinol (**5**); R = C_6H_4OH-*p*

The biosynthetic formulation of four resveratrol units undergoing phenol oxidative coupling *via* the resveratrol dimer, ε-viniferin is given in the Scheme.

Both ε-viniferin and vaticaffinol isolated in this study showed antibacterial activity towards Oxford *Staphylococcus* and *Escherichia coli* when tested by the filter paper disc method in the Mueller Hinton Agar medium.

After the separation of vaticaffinol, the silica-gel column when eluted with acetone–benzene (2:3) gave a further phenol, bergenin (**6**).



(6)

Experimental

The bark of *Vatica affinis*, collected from the Kanneliya rain forest, in the South of Sri Lanka was dried, chipped, and ground in a mill. The powdered bark (3.25 kg) was extracted with cold acetone in the dark for 7 days. The filtrate on concentration under reduced pressure yielded a pale brown solid (282 g, 8.67%). The extract (20.0 g) was separated on a column of silica gel. Elution with benzene gave a yellow oil containing mainly hydrocarbons and terpenes. For other details see ref. 4.

Isolation of the Resveratrol Dimer (ε-*Viniferin*).—The column when eluted with acetone–benzene (3:7) separated an off-white solid (124 mg, 0.05%). This was purified on silica-gel preparative (0.5 mm) plates (acetone–benzene 2:1) to afford an off-white solid (108 mg, 0.046%) which could not be crystallised. It was very hygroscopic, and gave an orange colour which subsequently turned to dark brown, then to green on a t.l.c. plate when sprayed with ceric sulphate; it had m.p. 146—148 °C (lit.,[2] m.p. 155—160 °C); M^+, 454.1415 (high resolution mass spectrometry) ($C_{28}H_{22}O_6$ requires M^+, 454.1416); $\lambda_{max.}$(in EtOH) 218 (log ε 4.53), 286sh (4.25), 311 (4.40), 324 (4.48), and 315sh (4.17) nm; $\lambda_{max.}$(ethanolic NaOH) 222 (4.43), 245 (4.37), 300 (3.92), and 328 (3.99) nm; $\nu_{max.}$(KBr) 3 230, 1 595, 1 510, 1 440, 1 330, 1 000, and 830 cm^{-1}; δ [$(CD_3)_2CO$, 60 MHz] 4.45 (1 H, d, *J* 6.0 Hz), 5.42 (1 H, d, *J* 6.0 Hz), 6.23 (3 H, s), 6.31 (1 H, d, *J* 2 Hz), 6.60—7.0 (m), 7.16 (3 H, d, *J* 2 Hz), and 7.30 (1 H, d, *J* 2 Hz); for ^{13}C n.m.r. results [$(CD_3)_2CO$] see Figure 1.

Methylation of ε-*Viniferin*.—ε-Viniferin (60 mg), potassium carbonate (500 mg), and dimethyl sulphate (5 drops) were refluxed with stirring in anhydrous acetone for 24 h. The mixture was filtered, evaporated, and the pure methyl ether was separated on preparative plates (using benzene) to yield a fluorescent amorphous solid (42 mg, 60.8%) (Found: M^+, 524.2215. $C_{33}H_{32}O_6$ requires M^+, 524.2199); $\nu_{max.}$(KBr) 2 915, 1 590, 1 505, 1 455, 1 295, 1 240, 1 110, 990, and 825 cm^{-1}; δ($CDCl_3$, 60 MHz) 3.73 (s, 2 × OCH_3), 3.76 (s, OCH_3), 3.80 (s, OCH_3), 3.86 (s, OCH_3) 4.50 (1 H, d, *J* 6 Hz), 5.50 (1 H, d, *J* 6 Hz), 6.40 (3 H, s), 6.50 (1 H, d, *J* 2 Hz), and 6.60—7.43 (m); *m/z* 524 (M^+, 14%), 403 (11), 279 (27), 227 (10), 218 (4), 213 (7), 167 (41), 150 (13), 149 (100), 141 (10), 140 (6), 135 (14), 129 (25), 125 (8), 121 (9), 115 (33), 113 (19), 112 (15), 111 (16), 109 (13), 107 (6), 99 (11), 97 (24), 95 (18), 85 (27), and 83 (34).

Isolation of Vaticaffinol.—The column when eluted with acetone–benzene (7:13) gave a pale brown solid containing vaticaffinol (8.1 g, 40.5%) as a mixture contaminated with bergenin. This was purified on several preparative plates to yield the pure vaticaffinol as an amorphous white solid.

Vaticaffinol could not be recrystallised but was obtained as a solid foam with a suitable solvent mixture (acetone–benzene, 3:2); m.p. 280—282 °C (decomp.), $[\alpha]_D^{25}$ −22.5° (MeOH); M^+, by accurate mass measurement was unsuccessful; $\lambda_{max.}$(MeOH) 288 nm (log ε 4.44) (no shift with sodium acetate and boric acid); $\nu_{max.}$(KBr) 3 200, 1 600, and 830 cm^{-1}; δ_H[$(CD_3)_2CO$, 360 MHz] 3.19 (1 H), 4.09 (1 H, dd), 4.44 (1 H, d), 4.55 (1 H, dd), 4.68 (1 H, d), 5.21 (1 H, d), 5.37 (1 H, d), 5.76 (1 H, d), and 6.04—7.60 (overlapping multiplets, 24 ArH). Decoupling experiments on irradiation at δ 5.76 (360 MHz) enabled the doublet at δ 4.44 to collapse to a singlet; irradiation at δ 5.37 enabled the doublet at δ 4.68 to collapse to a sharp singlet; irradiation at δ 5.21 changed the peak shape at δ 3.19; irradiation at δ 4.68 enabled the doublet at δ 5.37 to collapse to a singlet; irradiation at δ 4.44 enabled the doublet at δ 5.76 to collapse to a singlet; irradiation at δ 3.19 enabled the resonance at δ 5.21 to collapse to a singlet. For the ^{13}C n.m.r. results, δ_C[$(CD_3)_2CO$], see Figure 3; *m/z* parent molecular ion was not obtained; *m/z* 347 (18), 200 (19), 108 (29), 107 (46), 99 (60), 94 (100), 66 (27), and 65 (26).

Deca-O-methylvaticaffinol.—Vaticaffinol (500 mg) was refluxed in anhydrous acetone (30 ml) with dimethyl sulphate (1.0 ml) and potassium carbonate (1.50 g) for 36 h. The solution was filtered and the residue was repeatedly evaporated with benzene. The methyl ether when purified on a preparative plate, afforded a pale brown foam (386 mg, 66.8%), m.p. 160—162 °C, $[\alpha]_D^{25}$ +20.9° ($CHCl_3$) (Found: M^+, 1 046.4253 $C_{66}H_{62}O_{12}$ requires M^+, 1 046.4251); $\lambda_{max.}$($CDCl_3$) 225 (log ε 4.47), 245 (4.49), and 284 nm (4.21); $\nu_{max.}$(KBr) 2 910, 2 810, 1 600, 1 505, 1 455br, 1 300—1 240, 1 195, 1 160, 1 145, 1 125, 1 050, 920, and 830 cm^{-1}; δ($CDCl_3$, 270 MHz) 3.15 (s, OCH_3), 3.45 (1 H, d), 3.57 (s, OCH_3), 3.62 (s, OCH_3), 3.71, 3.75, 3.76, 3.77, 3.781, 3.784, 3.79 (together 7 × OCH_3), 4.08 (1 H, t), 4.25 (1 H, d), 4.43 (1 H, d), 4.50 (1 H, d), 5.09 (1 H, d), 5.38 (1 H, d), 5.84 (1 H, d), and 6.13—7.36 (overlapping multiplets ArH); δ_H($CDCl_3$, 360.13 MHz) 3.14 (s, OCH_3), 3.43 (1 H, d), 3.57 (s, OCH_3), 3.62 (s, OCH_3), 3.71 (s, OCH_3), 3.75 (s, OCH_3), 3.76 (s, OCH_3), 3.77 (s, OCH_3), 3.776 (s, OCH_3), 3.785 (s, OCH_3), 4.03 (1 H, t), 4.25 (d), 4.43 (d), 4.49 (d), 5.08 (d), 5.37 (d), 5.83 (d), 6.13—7.35 (complex multiplets, ArH); for ^{13}C n.m.r. results ($CDCl_3$, 67.89 MHz) see Figure 3; *m/z* 1 046 (M^+, 97%) 938 (100), 684(59), 654 (31), 538 (41), 523 (53), 522 (66), 469 (51), 415 (66), 403 (51), 390 (41), 387 (52), 270 (39), 268 (32), 257 (60), 227 (71), 197 (31), 169 (43), 151 (43), 139 (41), 135 (80), and 129 (36).

Deca-acetate of Vaticaffinol.—Vaticaffinol (215 mg) was acetylated with acetic anhydride (1.0 ml) and pyridine (3 ml) at room temperature for 24 h. The product after work-up and purification on preparative plates yielded the deca-acetate as a glassy solid (234 mg, 73.8%), m.p. 154—156 °C, $[\alpha]_D^{25}$ −33.9° ($CHCl_3$); $\nu_{max.}$(KBr) 2 905, 1 760, 1 600, 1 500, 1 425, 1 370, 1 190br, 1 120, 1 015, 910, 840, and 670 cm^{-1}; δ($CDCl_3$, 60 MHz) 1.63(s), 1.83(s), 2.27 (overlapping signals), 3.8—4.8 (complex signals), and 5.33—7.66 (overlapping multiplets).

Degradation of Vaticaffinol.—(a) *Alkali fusion.* Vaticaffinol (430 mg) was fused with NaOH (4 g), KOH (4 g), and water (1 ml) at 275 °C for *ca.* 15 min. The cooled melt was dissolved in water, acidified, and extracted with ether. The ether extract was re-extracted with aqueous sodium carbonate and the acidic fraction was obtained after neutralisation with dilute HCl. On concentration the acidic fraction gave a white solid which was purified on a preparative plate. The pure product recrystallised from water as white needles (38 mg), m.p. 212—213 °C (lit.,[6] 214—216 °C), and was identified as *p*-hydroxybenzoic acid by comparison with an authentic sample (m.p., mixed m.p., ^1H n.m.r. and co-t.l.c.).

(b) *With concentrated* HNO_3. Vaticaffinol (300 mg) and concentrated HNO_3 (10 ml) were heated on a water-bath at 100 °C for 1 h. The solution was diluted and extracted with ether. The ether extract was re-extracted with aqueous $NaHCO_3$ and the aqueous portion, after acidification, was extracted with ether. The acidic portion on concentration and recrystallisation from methanol afforded yellow needles (94 mg), m.p. 120—122 °C (lit.,[6] 122 °C), identified as picric acid by m.p., mixed m.p. and comparison with an authentic sample.

Oxidation of the Deca-O-methyl Ether.—The decamethyl ether of vaticaffinol (300 mg) was heated at 100 °C with conc. HNO_3 (15 ml) for 2 h. The solution was then diluted with water and extracted with ether. The acidic fraction of the ether extract was isolated with aqueous $NaHCO_3$. The aqueous portion after neutralisation with dilute HCl was re-extracted with ether. On concentration this afforded a mixture of acids (81 mg). By preparative t.l.c. the major acid was separated and identified as 3-nitroanisic acid, m.p. 182—184 °C (lit.,[6] 187 °C) (Found to be identical with a synthesized sample).

Isolation of Bergenin.—The extraction of the powdered bark (3.25 kg) of *V. affinis* with cold acetone deposited a white solid on the walls of the flask. The extract was concentrated and the precipitate filtered off. The precipitate after washing repeatedly with cold acetone and on recrystallisation from methanol yielded bergenin as white crystalline plates (40 g, 0.13%), m.p. 142—144 °C (hydrated) and 232—234 °C (anhydrous) (lit.,[6] 138—139 °C, solidifying and remelting at 230 °C).

Bergenin was also separated from the column when eluted with acetone–benzene (2:3). It is insoluble in low polar solvents and in acetone but soluble in hot methanol and water. It gave a green colour with iron(III) chloride solution; $\lambda_{max.}$(MeOH) 271 (log ε 3.79) and 310 nm (3.39); $\lambda_{max.}$(methanolic NaOH) 288 (3.63) and 346 nm (3.36). No change with NaOAc–H_3BO_3; $\nu_{max.}$(KBr) 3 400—3 200, 1 700, and 1 610 cm^{-1}. δ_H[$(CD_3)_2SO$, 60 MHz] 8.38 (OH), 7.03 (1 H, s), 5.5 (br, OH), 5.01 (2 H, d, *J* 10 Hz), 3.83 (3 H, s, OCH_3), and 3.4—4.0 (br, complex signals).

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