

Identification of two stilbenoids from *Vitis* roots

by

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S u m m a r y : Two oligostilbenes were isolated from roots of *Vitis vinifera* cv. Chardonnay and their structures were identified. One compound, ampelopsin A, is a dimer of resveratrol and has been found previously only in the roots of *Ampelopsis brevipedunculata* var. *hancei* (Vitaceae). The second, hopeaphenol, can be regarded as a dimer of ampelopsin A; its presence in Vitaceae is reported here for the first time. Both compounds are present in mg per g levels in vine roots.

K e y w o r d s : Vitaceae, *Vitis* species, vine roots, oligostilbenes, ampelopsin A, hopeaphenol.

Introduction

This work is part of a systematic investigation of stilbenoids in vine roots, a family of highly bioactive compounds which possibly play a role in plant protection. In previous papers we reported the identification of four oligostilbenes bearing one double bond between C-7' and C-8' (MATTIVI and RENIERO 1992; KORHAMMER *et al.* 1995). The 2 stilbenoids reported here (Figure), ampelopsin A (**1**) and hopeaphenol (**2**), can be considered - formally at least - as resveratrol derivatives, whose structures do not con-

tain any non-aromatic double bond as a consequence of the formation of a 7-membered ring. The isolation of **1** and **2** was carried out on material from *Vitis vinifera* but their presence was also confirmed in many other *Vitis* species and hybrids.

Material and methods

Extraction and purification: A double extraction with methanol (7 l) was carried out starting from 500 g of fresh roots of *V. vinifera* cv. Chardonnay. The vines were one-year-old plants grown in pots in greenhouses of the Istituto Agrario di S. Michele all'Adige (Italy). The solvent was evaporated under vacuum and the residue dissolved in EtOAc. The hydrophilic fraction was removed with NaCl satd. water. The EtOAc fraction was purified by absorption onto Amberlite XAD-2 (MATTIVI and RENIERO 1992). Stilbene oligomers were subsequently desorbed with EtOAc. The solvent was evaporated and the residue dissolved in the lowest possible amount of MeOH. Isolation was performed by multiple injection of 90 µl aliquots on a semi-preparative HPLC system (isocratic, water: CH₃CN 55:45 v/v at 1.6 ml/min; column: Lichrospher 100 RP-18, 25x1 cm, particle size 10 µm). Approx. 1 ml fractions were collected between 5 and 25 min, yielding a total of ca. 1.6 g of **1** and 2.3 g of **2**.

The presence of these compounds was subsequently tested and confirmed by an HPLC-DAD method (MATTIVI *et al.* 1996) for *V. vinifera* (cvs Riesling, Müller Thurgau, Bonarda and Barbera), *V. Longii*, *V. Berlandieri*, *V. cinerea*, *V. Solonis Longii*, *V. Solonis Richter*, *V. rupestris* and *V. riparia*, and in over 20 different hybrids of them. Pure species, grown in the field, originated from the *Vitis* species collection of the Istituto Agrario di S. Michele while potted hybrids were obtained from the experimental field of the University of Milan, located near Arcagna.

N M R d e t e r m i n a t i o n : NMR data were recorded on a BRUKER AMX 500 at 500.13 MHz for

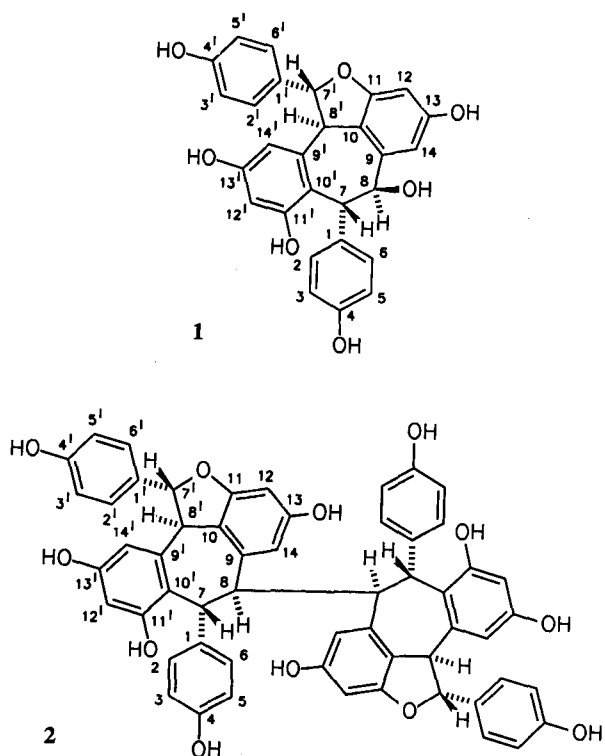


Figure: Chemical structures of ampelopsin A (**1**) and hopeaphenol (**2**).

^1H NMR or at 125.77 MHz for ^{13}C NMR. Measurements were performed using a solution of ca. 15 mg of compound **1** or **2** for the recording of ^1H NMR and the double quantum filtered COSY spectra and a solution of 100 mg (**1**) or 80 mg (**2**) in 0.5 ml of solvent for the recording of ^{13}C NMR, HMQC, HMBC, and NOE difference spectra. For all spectra a dual $^1\text{H}/^{13}\text{C}$ probe with a 5 mm bore was used. All experiments were performed at 295 K. Chemical shifts (δ) are quoted in ppm relative to TMS with the methyl group of acetone- d_6 as the reference (2.04 for ^1H and 29.8 for ^{13}C). Coupling constants (J) are quoted in Hz and have been rounded to the next 0.5 Hz.

Spectroscopical data for **1**: NMR data in the Table, MS (FAB): $m/z = 470$ (M^+ ; 3 %), 452 ($\text{M}^+ - \text{H}_2\text{O}$; 10 %), UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 283 (3.84).

Spectroscopical data for **2**: NMR data in the Table, MS (FAB): $m/z = 453$ ($\text{M}^+/2$; 9 %), 452 ($\text{M}^+/2 - \text{H}$; 18 %), UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 283 (4.20). Additional UV data see MATTIVI and RENIERO (1996).

Results and discussion

The ^1H NMR of compound **1** shows 5 signals in the aliphatic region, one AX system with shifts at 4.13 and

5.74 ppm and an AB system (5.39, 5.42 ppm) of which one signal is broadened due to coupling to a D_2O exchangeable peak. The aromatic region shows 2 pairs of doublets with a coupling constant of 2 Hz, indicating meta-coupled protons, and 2 sets of multiplets deriving from *ortho*-substituted phenol derivatives. At the low field region of the spectrum 5 D_2O exchangeable peaks can be found, obviously deriving from phenolic OH groups. The C-C connectivity was established by recording a ^1H - ^1H COSY, an inverse ^1H - ^{13}C (HMQC) shift correlation, and an inverse ^1H - ^{13}C shift correlation using long range couplings (HMBC). The MS spectrum (FAB) showed a molecular ion at $m/z = 470$ ($\text{C}_{28}\text{H}_{22}\text{O}_7$) and a $[\text{M}^+ - \text{H}_2\text{O}]$ signal at $m/z = 452$, strongly suggesting a resveratrol dimer with one additional molecule of water. Combination of these data led to a proposal of structure **1** for compound **1**. The relative stereochemistry of **1** could be proved by NOE difference spectra and turned out to be *trans* for each set of aliphatic resonances (Enhancements: $\text{H8}-\text{H}[2,6] = 18.8\%$; $\text{H8}'-\text{H}[2,6]' = 14.6\%$) and *trans* for $\text{H8}'$ and H7 (Enhancements: $\text{H8}'-\text{H}[2,6] = 4.6\%$). The proposed formula is identical with the known structure of ampelopsin A (OSHIMA *et al.* 1990), an oxidative dimer of resveratrol, previously found only in the roots of *Ampelopsis brevipedunculata*

Table

^1H and ^{13}C NMR spectral data of ampelopsin A and hopeaphenol

Ampelopsin A			Hopeaphenol		
C,H	^{13}C NMR	^1H NMR	C,H	^{13}C NMR	^1H NMR
7	43.9	5.42 (d, $J=5$)	7	41.2	5.80 (br s)
8'	49.5	4.13 (d, $J=11.5$)	8	48.2	3.91 (br s)
8	71.2	5.39 (dd, $J=5, J=5$)	8'	49.7	4.22 (d, $J=12$)
7	88.5	5.74 (d, $J=11.5$)	7'	88.2	5.75 (d, $J=12$)
12	97.1	6.13 (d, $J=2$)	12	95.2	5.73 (d, $J=2$)
12'	101.4	6.40 (d, $J=2$)	12'	101.2	6.52 (d, $J=2$)
14'	105.5	6.21 (d, $J=2$)	14'	106.3	6.28 (d, $J=2$)
14	110.3	6.58 (d, $J=2$)	14	111.2	5.16 (d, $J=2$)
3,5	115.5	6.61 (m)	3,5	115.3	6.56 (m)
3',5'	116.0	6.75 (m)	3',5'	116.0	6.78 (m)
10,10'	118.4, 118.9		10	118.6	
2,6	128.8, 128.9	6.87 (m)	10'	121.2	
2',6'	129.9, 130.0	7.09 (m)	2,6	129.4	6.88 (m)
1'	131.0		2',6'	130.3	7.12 (m)
1	132.7		1'	131.0	
9	140.5		1	135.2	
9'	143.1		9	140.4	
4	156.1		9'	142.4	
13'	157.3		4	155.6	
13	158.5		11',13'	157.1, 157.2	
11',4'	159.0		4',11,13	158.4, 158.7, 159.2	
11	160.2				
OH		8.06 (s)			7.40 (s)
OH		8.11 (s)			7.96 (s)
OH		8.24 (s)			8.18 (s)
OH		8.30 (s)			8.45 (s)
OH		8.44 (s)			8.51 (s)

var. *hancei* (Vitaceae). It should be noted that the structure of this compound closely resembles that of the tetramer r-2-viniferin (KORHAMMER *et al.* 1995) as well as that of balanocarpol (DIYASENA *et al.* 1985), both C-7 stereoisomers.

The ^1H NMR spectrum of compound **2** showed strong similarities to **1**: 4 resonances in the aliphatic region, in the aromatic region 4 doublets with a coupling constant of ca. 2 Hz indicating 2 pairs of meta-coupled protons and 2 pairs of strongly coupled signals indicating the presence of two *ortho*-substituted phenol rings. As with compound **1** a set of 5 D_2O exchangeable signals at the low field end of the spectrum could be found. By employing the same method as for **1** (^1H - ^1H COSY, ^1H - ^{13}C HMQC, ^1H - ^{13}C HMBC), we found a molecular structure identical to **1** with an unknown substituent at C8. The D_2O exchange experiments, however, proved the absence of any aliphatic hydroxy group. Additionally, the coupling constants for the protons in the 7-membered ring differed significantly from **1** ($J < 1$ Hz), so either the stereochemistry regarding H7 and H8 was different or the molecule geometry was distorted by the substituent at C8. NOE difference spectra proved the relative stereochemistry of **2** as being identical to **1** (Enhancements: H8-H[2,6] = 8.2 %; H8'-H[2',6'] = 8.9 %, H8'-H[2,6] = 2.2 %). MS analysis (FAB) showed strong signals at $m/z = 452$ and 453 , respectively, which we could not superimpose, regarding the ^1H NMR data, with a simple dimeric structure. Thus structure **2**, a dimer of dimers linked at C8, was proposed for **2**. This structure and all the data obtained are in agreement with the known stilbenoid hopeaphenol ($\text{C}_{56}\text{H}_{42}\text{O}_{12}$) (KAWABATA *et al.* 1992). This was the first natural resveratrol tetramer described, and its structure was determined by x-ray methods (COGGON *et al.* 1966). The compound has been found so far in some Dipterocarpaceae, in particular in the heartwood of *Balanocarpus heimii* and *Hopea odorata* (COGGON *et al.* 1965), *Shorea talura* and *S. robusta* (MADHAV *et al.* 1967), in the leaves and roots of *Vateria indica* (DAYAL 1987) and moreover in the roots of *Carex pumila* (Cyperaceae) (KAWABATA *et al.* 1992) and *Sophora leachiana* (Leguminosae) (IINUMA *et al.* 1994). Its presence in Vitaceae is reported here for the first time.

Preliminary HPLC screening of the above mentioned *Vitis* species and hybrids showed that both compounds are present in large quantities. Ampelopsin A has been found in all the roots tested, at a level between 2 and 16 mg/g of

fresh root. Hopeaphenol was also common in the large majority of genotypes analyzed, while it was absent in a few others. Its concentration was always lower than that of ampelopsin A, varying between 0.5 and 8 mg/g of fresh root. More detailed analytical data on these and other compounds of the same family will be presented later.

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