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AN OLIGOSTILBENE FROM *VITIS* ROOTS*

SIEGFRIED KORHAMMER,† FABIANO RENIERO†‡ and FULVIO MATTIVI‡§

†Commission of the European Communities, Joint Research Centre, Environment Institute, I-21020, Ispra, Italy; ‡Istituto Agrario, Dipartimento Laboratorio Analisi e Ricerche, I-38010, S. Michele all'Adige, Italy

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*IN MEMORY OF HILMAR SPIESECKE WHO DIED IN JULY 1993

Key Word Index—*Vitis*; Vitaceae; vine roots; oligostilbenes; r-2-viniferin; resveratrol; isochronous double bond.

Abstract—Isolation and structural identification of a new tetramer of resveratrol (3,5,4′-trihydroxystilbene), r-2-viniferin, from the roots of species and hybrids of the genus *Vitis* is reported.

INTRODUCTION

This work is part of a systematic investigation of stilbenoids present in vine roots, for a subsequent assessment of their biochemical role. In a previous paper [1] we reported on the identification of three oligostilbenes from the vine roots. Two of the compounds, ε-viniferin and gnetin H, are already known in the literature. The first one is a dimer, recognized as a 'stress metabolite' in the leaves of *Vitis* plants, where it was first isolated. The second is a trimer, previously found in the wood of *Welwitschia mirabilis* (Gnetatae). The third compound is a newly identified tetrastilbene present in large quantities as the main free phenolic in the roots of *Vitis*, and it was named r-viniferin (**1**). The novel compound we present in this paper is also a tetramer, named r-2-viniferin (**2**) and it is related to **1**.

RESULTS AND DISCUSSION

The mass spectrum showed an $[M]^+$ ion at m/z 906, which together with the data from ^{13}C and ^1H NMR spectra (Table 1), allowed us to deduce the molecular formula $C_{56}H_{42}O_{12}$. The ^1H NMR spectra showed signals integrating for 10 hydrogens between δ7.7 and 8.7. By acetylation a deca-acetate was obtained, whose IR and ^1H NMR spectra did not show signals caused by hydroxyls, thus confirming the presence of 10 phenolic hydroxyl groups in the original compound. The UV spectra suggested the presence of a *trans* stilbene double bond with a bathochromic shift of 6.6 nm with respect to the band I of **1**, in agreement with the presence of an extra free *p*-hydroxyl instead of an ether group [2]. Measurements performed in sodium ethoxide–ethanol showed a very short shift and a lowering of band I that did not provide additional information on the position of the hydroxyl groups.

Two carbon atoms (δ122.7 and 131.0) were connected to a signal in the ^1H spectrum at δ6.4, integrating for two protons. This proton resonance showed neither a ^3J nor a long-range coupling. After addition of benzene-d_6 to the solvent acetone-d_6 (acetone–benzene *ca* 1 : 1), the isochronous (accidentally overlapping) signals split into an AB spin system of the expected double bond, δ6.61 (δ_C131.1) and $\delta = 6.68$ (δ_C122.8), with $^3J = 19$ Hz, confirming a *trans* configuration. The presence of two 2,3-diaryl-2,3-dihydrobenzofuran rings, three 1,4-disubstituted benzene rings, one 1,3,5-trisubstituted benzene ring, three 1,2,3,5-tetrasubstituted benzene rings and one 1,3,4-trisubstituted benzene ring were deduced on the basis of the data reported in Table 1.

A seven-carbon ring was also present, with two α aliphatic hydrogens, H-7″ and H-8″, whose ^3J value (3.5 Hz) and homonuclear dipolar couplings were in agreement with a *Z* configuration, similar to that proposed for balanocarpol (Dipterocarpaceae) [3]. The same ring in *E* configuration was also found in other molecules like hopeaphenol (Dipterocarpaceae) [4, 5], and ampelopsin A (Vitaceae) [6]. The results of the 1D- and 2D-NOE-measurements indicate the *E* configuration of H-7, H-8 and H-7‴, H-8‴. The different ^3J coupling constants, 5.3 and 11.5 Hz, respectively, could be explained with the widening of the angle between H-7‴ and H-8‴ owing to the seven-membered ring and, therefore, an increasing of the coupling constant. From the above evidence, structure**2** is proposed for the new oligostilbene.

The bond between C-3′ and C-8″ can be formally regarded as a linkage of a balanocarpol with an ε-viniferin unit. The involvement of the C-3′ atom in the formation of a C–C bond in resveratol oligomers has, as far as we know, never been reported to date except for **1**. This

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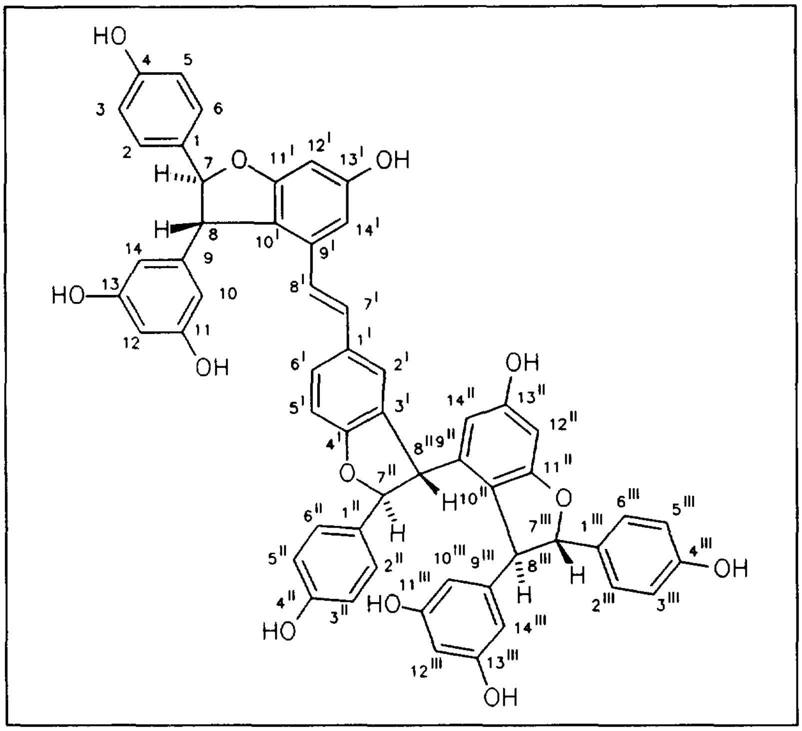
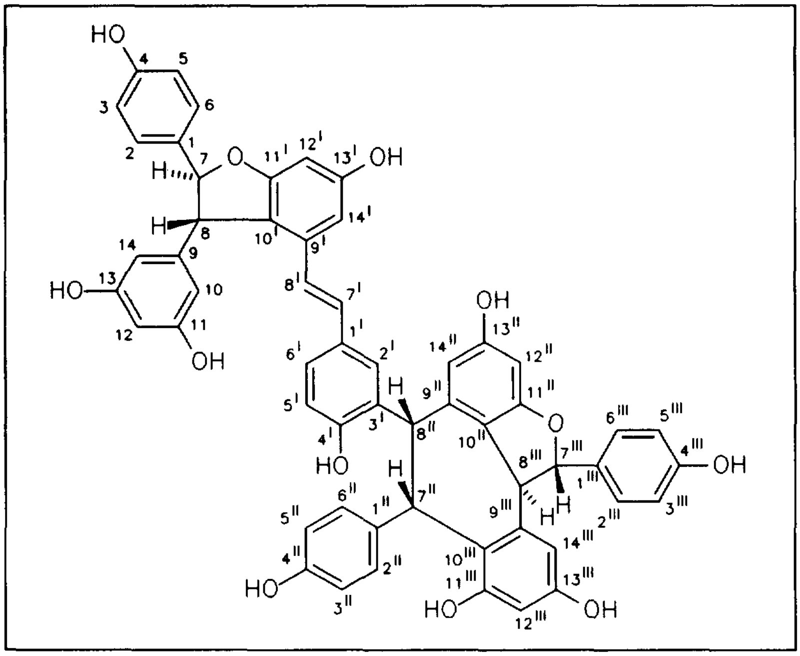
§Author to whom correspondence should be addressed.



1



2

peculiarity let us to hypothesize **1** to be the precursor for the biosynthesis of **2** via acid-catalysed opening of a dihydrobenzofuran ring. This hypothesis is supported by the experimental evidence that **2** can be easily obtained in acidic media (HCl 0.1% in MeOH at room temperature) from **1**.

EXPERIMENTAL

The isolation of **2** from grapevine roots was carried out on the hybrid 44–53, but it was also found in all the other *Vitis* species and hybrids tested [1]. Phenolic compounds were cold-extracted from 100 g of frozen roots, by MeOH

Table 1. ^1H and ^{13}C NMR spectral data of r-2-viniferin

C,H	^{13}C	^1H	J(Hz)	C-H l.r.*	H-H l.r.*	NOE
1	133.9			6.84,4.41		
2,6	127.9	7.20	8.7	5.37	5.37	4.41,5.37
3,5	116.2	6.84	8.7		5.37	
4	157.9^a					
7	93.9	5.37	5.3	4.41,7.20	7.20,6.84	7.20,6.17
8	57.1	4.41	5.3	5.37,6.17	6.17,6.27	6.17,6.40,7.20
9	147.2			5.37,4.41		
10,14	106.9	6.17	2.2	4.41,6.23	4.41	4.41,5.37,6.40
11,13	159.8					
12	102.2	6.23	2.2	6.17	6.17	
1′	129.0			6.70,6.40		
2′	132.5	6.10	2.3	6.88,6.40		6.40
3′	132.8			6.70		
4′	155.2			6.88		
5′	115.5	6.70	8.4			
6′	123.7	6.88	8.4,2.3	6.40	6.10	6.40
7′	122.7	6.40			6.53	4.41,6.10,6.17 6.53,6.88
8′	131.0	6.40			6.53	
9′	136.6			6.40,4.41		
10′	119.0			4.41,6.53		
11′	162.5			4.41		
12′	96.6	6.27	2.3	6.53	4.41	
13′	158.2^a					
14′	104.5	6.53	2.3	6.40		6.40
1″	135.4			6.67,5.40		
2″,6″	128.8	7.04	8.6	5.40	5.40	5.49,5.40
3″,5″	115.5	6.67	8.6		5.40	
4″	156.0			7.04,6.67		
7″	40.7	5.40	3.5	7.04	6.67,7.04	7.04
8″	41.3	5.49	3.5	5.40,6.06	4.25	5.40,6.06,7.04
9″	141.2					
10″	120.4					
11″	158.5^a					
12″	96.1	6.10	2.1	6.06		
13″	158.8^a					
14″	110.1	6.06	2.1	6.10		5.49
1‴	131.1			6.78,5.89,4.25		
2‴,6‴	130.1	7.15	8.7	5.89	5.89	4.25,5.89
3‴,5‴	116.0	6.78	8.7		5.89	
4‴	159.6^a					
7‴	88.5	5.89	11.5	7.15,4.25	6.27,6.78,7.15	6.27,7.15
8‴	49.5	4.25	11.5	5.89,6.27	6.03,5.49,6.27	7.15
9‴	142.3			5.89,4.25		
10‴	120.2			4.25,6.03,5.40,6.27		
11‴	160.3^a					
12‴	101.0	6.03	2.0	6.27	4.25	
13‴	157.0			6.03,6.27		
14‴	105.0	6.27	2.0	6.03	4.25,5.89	5.89,7.15

^{a}Exchangeable
*l.r. = Long range.

(2 l), in N_2 and in the dark. The methanol extract was evapd to low volume (100 ml) on a rotating evaporator (35°), diluted 10 times with H_2O, and loaded (10 runs) on a column (30 × 3 cm) of styrene-divinylbenzene resin (Amberlite XAD-2, particles size mix. 0.1–0.25 mm), slurry-packed in MeOH and purified through the sequence of solvents (250 ml of each) MeOH, CH_2Cl_2, Me_2CO, MeOH, and finally H_2O (700 ml). The extract loaded on top of the column was washed with H_2O (700 ml) as well as with pentane–CH_2Cl_2 (2:1) mixt. (850 ml). Oligostilbenes were finally eluted with EtOAc (500 ml). The isolation of the studied compound was performed by

semiprep. HPLC using a LiChrospher 100 RP-18 column (10 μm particle size, E.Merck, column dimensions 25 × 1 cm), eluting with a MeCN–H_2O mixt. (linear gradient from 30% MeCN to 50% MeCN in 40 min, with a flow rate of 2.0 ml min^{-1}). ^1H NMR spectra were taken at 400 and 500 MHz, and ^{13}C NMR spectra at 100 and 125 MHz, in Me_2CO-d_6. The resonances of the methyl group of Me_2CO-d_6 were used as references for δ values: $\delta_H = 2.04$; $\delta_C = 29.8$ FAB-MS were obtained in negative mode, using a glycerol matrix. UV spectra were recorded both in absolute EtOH and with addition of Na ethylate [2]. IR spectra were recorded in KBr. Acetylation was performed under the conditions described in ref. [7].

r-2-Viniferin (**2**). Amorphous solid, yellowish. Mp: > 300°. ^1H and ^{13}C NMR: Table 1. $[\alpha]_D^{25}$ + 132.9 (MeOH; *c* 1.1). Found: C, 73.84, H, 4.60, $C_{56}H_{42}O_{12}$ requires C, 74.17, H, 4.63%. IR ν cm^{-1}: 3385, 1696, 1601, 1513, 1486, 1444, 1366, 1341, 1232, 1173, 1145, 1129, 1005, 833. UV λ_{max}^{EtOH} nm (log ε): 329.0 (4.32), 285.4 (4.30), 224.0sh (4.87). UV $\lambda_{max}^{EtOH/NaOEt}$ nm (log ε): 329.4 (4.27), 294.0 (4.36), 243.4 (4.77).

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