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A novel oligostilbene named (+)-viniferol A from the stem of *Vitis vinifera* 'Kyohou'

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Abstract—A novel stilbenetetramer named (+)-viniferol A was isolated from the stem of *Vitis vinifera* 'Kyohou' and the structure was elucidated on the basis of the spectral evidence.

1. Introduction

In previous papers, we reported the isolation and structures of oligostilbenes from the corks of *Vitis vinifera* 'Kyohou' cultivated in Wakayama Prefecture, Japan.[1] Our further study on the constituents of the above plant led to the isolation of a novel oligostilbene named (+)-viniferol A together with several known compounds including stilbene glycoside. In this paper, we describe the isolation and structural elucidation of the oligostilbenes from the stem of *V. vinifera* 'Kyohou'.

2. Results and discussion

2.1. Isolation

The methanol extract of the stem of *V. vinifera* 'Kyohou' was successively partitioned between water and *n*-hexane, chloroform, ethyl acetate and *n*-butanol to give the corresponding solubles, respectively. The ethyl acetate soluble fraction was fractionated by repeated medium-pressure column chromatography (MPCC) using silica-gel and ODS columns and preparative HPLC to give (+)-viniferol A (**1**) from the chloroform–methanol (4:1) eluate of the first silica-gel column (Fig. 1). On the other hand, the acetone extract of the stem of this plant gave (+)-ε-viniferin,[2] (+)-vitisin A,[3] (+)-ampelopsin A,[4] (+)-ampelopsin F,[5] (+)-hopeaphenol,[6,7] (−)-isohopeaphenol,[7] (−)-malibatol A,[8] (+)-resveratrol 10-*C*-glucoside[9] and (−)-resveratrol 11-*O*-glucoside[10] by repeated silica-gel column chromatography and MPCC using ODS column.

2.2. Structure of (+)-viniferol A

(+)-Viniferol A (**1**), $[\alpha]_D$=+136.6° (*c* 0.57, MeOH) was found to have the molecular formula $C_{56}H_{42}O_{12}$ determined by high-resolution FABMS. The ^{1}H NMR spectrum in acetone-d_6 of **1** exhibited signals for four sets of AA′XX′type (1,4-disubstituted) aromatic hydrogens at δ 6.85 and 6.64 (each 2H, d, *J*=8.4 Hz); 6.59 and 6.49 (each 2H, brs); 6.73 and 6.49 (each 2H, d, *J*=8.4 Hz); 7.25 and 6.79 (each 2H, d, *J*=8.4 Hz),[11] two sets of *meta*-coupled aromatic hydrogens δ 6.061 and 6.49 (each 1H, d, *J*=2.2 Hz); 5.95 and 5.52 (each 1H, d, *J*=2.2 Hz), and one set of AX_2-type *meta*-coupled aromatic hydrogens δ 6.11 (2H, d, *J*=2.2 Hz) and 6.15 (1H, t, *J*=2.2 Hz) as shown in Table 1. By the comparison of the signal patterns on ^{1}H NMR spectra of ε-viniferin and other oligostilbenes, the signals for two sets of aliphatic hydrogens at δ 5.66 and 4.30 (each 1H, d, *J*=12.8 Hz); 5.37 and 4.63 (each 1H, d, *J*=8.8 Hz) suggested the presence of two dihydrobenzofuran moieties bearing 4-oxyphenyl and 3,5-dioxyphenyl groups characteristic of oligostibenes derived from resveratrol molecule. The four aliphatic hydrogens coupled each other at δ 5.39 (1H, brs, H-7c), 3.99 (1H, brd, *J*=7.0 Hz, H-8c), 3.18 (1H, dd, *J*=7.0, 11.0 Hz, H-8b) and 3.23 (1H, d, *J*=11.0 Hz, H-7b) suggested the presence of a bicyclo-[6.3.0]undecane ring system. This was further confirmed by the following evidence. As shown in Fig. 2, the correlations of the HMBC spectrum were observed between H-8a and C-9a, C-14a, between H-7c and C-10a, C-11a, C-1c, C-2c (6c), C-8b, C-8c, C-9c, between H-8c and C-10a, C-7b, C-1c, C-7c, C-9c, C-14c, between H-8b and C-1b, C-9b, C-14b, C-8c, and between H-7b and C-1b, C-2b (6b), C-8b, respectively. The partial structure (C-10c to C-14d) was characterized by comparison of the ^{13}C NMR data of **1** with those of (+)-ε-viniferin (**2**). As shown in Table 2, the chemical shift values of C-10c to C-14d of **1** are closely similar to the corresponding values of **2** except for those of C-13c and C-14c.

Keywords: *Vitis vinifera* 'Kyohou'; oligostilbene; resveratrol tetramer.

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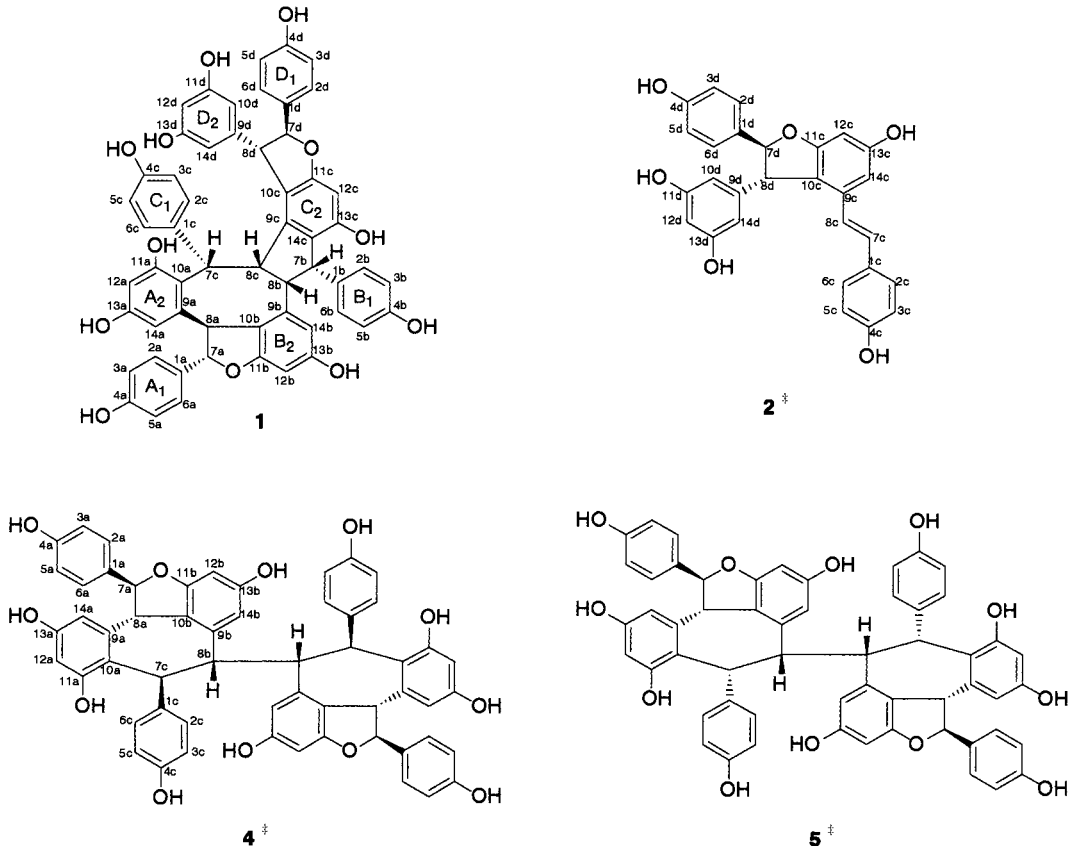

Figure 1. Structures of **1**, **2**, **4** and **5**.‡

Table 1. ^{1}H NMR data of (+)-viniferol A (**1**), (+)-ϵ-viniferin (**2**), (+)-hopeaphenol (**4**) and (−)-isohopeaphenol (**5**) in acetone-d_6

Positions	1	2	4	5
2a,6a	6.85 (2H, d, *J*=8.4 Hz)		7.09 (4H, d, *J*=8.8 Hz)	7.52 (4H, d, *J*=8.8 Hz)
3a,5a	6.64 (2H, d, *J*=8.4 Hz)		6.72 (4H, d, *J*=8.8 Hz)	6.72 (4H, d, *J*=8.8 Hz)
7a	5.66 (1H, d, *J*=12.8 Hz)		5.80 (2H, d, *J*=12.5 Hz)	5.56 (2H, d, *J*=10.3 Hz)
8a	4.30 (1H, d, *J*=12.8 Hz)		4.12 (2H, d, *J*=12.5 Hz)	5.37 (2H, d, *J*=10.3 Hz)
12a	6.061 (1H, d, *J*=2.2 Hz)		6.36 (2H, d, *J*=2.2 Hz)	6.32 (2H, d, *J*=2.2 Hz)
14a	6.49 (1H, d, *J*=2.2 Hz)		6.21 (2H, d, *J*=2.2 Hz)	6.19 (2H, d, *J*=2.2 Hz)
2b,6b	6.59 (2H, br s)			
3b,5b	6.49 (2H, br s)			
7b	3.23 (1H, d, *J*=11.0 Hz)			
8b	3.18 (1H, dd, *J*=7.0, 11.0 Hz)			
12b	5.95 (1H, d, *J*=2.2 Hz)		5.73 (2H, d, *J*=2.2 Hz)	5.79 (2H, d, *J*=2.2 Hz)
14b	5.52 (1H, d, *J*=2.2 Hz)		5.07 (2H, d, *J*=2.2 Hz)	5.42 (2H, d, *J*=2.2 Hz)
2c,6c	6.73 (2H, d, *J*=8.4 Hz)	7.17 (2H, d, *J*=8.4 Hz)	6.89 (4H, d, *J*=8.8 Hz)	6.33 (4H, d, *J*=8.8 Hz)
3c,5c	6.49 (2H, d, *J*=8.4 Hz)	6.73 (2H, d, *J*=8.4 Hz)	6.54 (4H, d, *J*=8.8 Hz)	6.30 (4H, d, *J*=8.8 Hz)
7c	5.39 (1H, br s)	6.90 (1H, d, *J*=16.5 Hz)		
8c	3.99 (1H, br d, *J*=7.0 Hz)	6.71 (1H, d, *J*=16.5 Hz)		
12c	6.063 (1H, s)	6.32 (1H, d, *J*=2.2 Hz)		
14c		6.72 (1H, d, *J*=2.2 Hz)		
2d,6d	7.25 (2H, d, *J*=8.4 Hz)	7.20 (2H, d, *J*=8.4 Hz)		
3d,5d	6.79 (2H, d, *J*=8.4 Hz)	6.82 (2H, d, *J*=8.4 Hz)		
7d	5.37 (1H, d, *J*=8.8 Hz)	5.42 (1H, d, *J*=5.5 Hz)		
8d	4.63 (1H, d, *J*=8.8 Hz)	4.47 (1H, d, *J*=5.5 Hz)		
10d,14d	6.11 (2H, d, *J*=2.2 Hz)	6.23 (2H, s)		
12d	6.15 (1H, t, *J*=2.2 Hz)	6.23 (1H, s)		

The stereochemistry of **1** was determined on the basis of ^{1}H NMR spectral data, as follows. The coupling constant (*J*=12.8 Hz) of H-7a and H-8a suggested the stereochemistry between H-7a and H-8a to be *trans*, as shown in the case of (+)-ampelopsin A (**3**) (*J*=11.7 Hz)[4] and (+)-hopeaphenol (**4**) (*J*=12.5 Hz).[6,7] The chemical shift value of H-8a (δ 4.30) indicated the stereochemistry between H-8a and H-7c to be *anti*, as shown in the case of

‡ Position numbers of ϵ-viniferin (**2**), hopeaphenol (**4**) and isohopeaphenol (**5**) are not same as those of reported. On these structures, they are re-numbered so as to correspond to the positions of (+)-viniferol A (**1**).

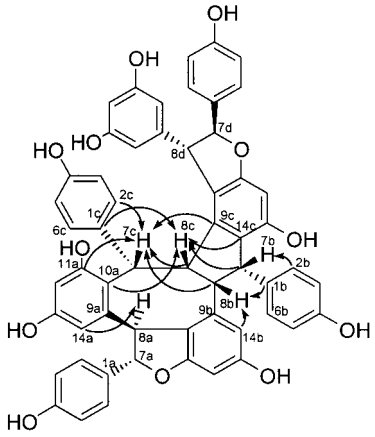

Figure 2. Important HMBC singals of (+)-viniferol A (**1**).

Table 2. ^{13}C NMR data of (+)-viniferol A (**1**), (+)-ϵ-viniferin (**2**), (+)-hopeaphenol (**4**) and (−)-isohopeaphenol (**5**) in acetone-d_6

Positions	1	2	4	5
1a	130.0 (s)		131.0 (s)	133.8 (s)
2a,6a	130.6 (d)		130.5 (d)	131.2 (d)
3a,5a	115.5 (d)		116.2 (d)	116.2 (d)
4a	158.4 (s)		158.8 (s)	159.1 (s)
7a	88.8 (d)		89.0 (d)	94.6 (d)
8a	50.5 (d)		49.9 (d)	54.2 (d)
9a	140.4 (s)		142.5 (s)	141.1 (s)
10a	120.3 (s)		122.0 (s)	118.8 (s)
11a	158.4 (s)		159.4 (s)	160.6 (s)
12a	101.2 (d)		101.0 (d)	102.7 (d)
13a	157.4 (s)		157.2 (s)	158.3 (s)
14a	105.9 (d)		106.3 (d)	107.4 (d)
1b	134.3 (s)			
2b,6b	129.0 (s)			
3b,5b	116.0 (d)			
4b	160.6 (s)			
7b	55.7 (d)			
8b	65.1 (d)			
9b	138.4 (s)		141.2 (s)	142.3 (s)
10b	121.7 (s)		119.6 (s)	117.7 (s)
11b	160.6 (s)		159.3 (s)	159.2 (s)
12b	96.5 (d)		95.3 (d)	95.3 (d)
13b	157.4 (s)		157.0 (s)	156.9 (s)
14b	112.6 (d)		111.8 (d)	110.2 (d)
1c	136.1 (s)	130.0 (s)	136.0 (s)	138.2 (s)
2c,6c	128.2 (d)	127.9 (d)	129.7 (d)	130.1 (d)
3c,5c	115.5 (d)	116.1 (d)	115.3 (d)	114.9 (d)
4c	155.8 (s)	158.1 (s)	155.5 (s)	155.1 (s)
7c	41.4 (d)	123.4 (d)		
8c	49.3 (d)	129.8 (d)		
9c	146.0 (s)	136.3 (s)		
10c	119.1 (s)	119.8 (s)		
11c	161.2 (s)	162.4 (s)		
12c	96.2 (d)	96.7 (d)		
13c	153.5 (s)	159.5 (s)		
14c	124.8 (s)	104.1 (d)		
1d	133.4 (s)	133.8 (s)		
2d,6d	129.0 (d)	128.7 (d)		
3d,5d	115.9 (d)	116.2 (d)		
4d	158.2 (s)	158.1 (s)		
7d	94.7 (d)	93.8 (d)		
8d	57.7 (d)	57.0 (d)		
9d	145.1 (s)	147.4 (s)		
10d,14d	107.5 (d)	106.9 (d)		
11d,13d	159.5 (s)	159.8 (s)		
12d	101.9 (d)	102.0 (d)		

(+)-hopeaphenol (**4**) (δ 4.12).[6,7,12,14] It caused such an upfield shift that H-8a is held in the shielding region of the aromatic group (C_1) at C-7c. The stereochemistries of H-8c, H-8b, H-7b and H-8d of **1**, respectively, were determined by the difference NOE experiments. Irradiation of H-7c showed enhancement of the signals of H-8c (3.4%) and H-8d (4.5%). Irradiation of H-8c showed enhancement of the signal of H-7b (2.6%). Irradiation of H-8b showed enhancement of the signals of H-8c (6.0%) and H-7b (1.9%). These data suggested the stereochemistries between H-7c and H-8c, between H-8c and H-8b, between H-8b and H-7b, and between H-7c and H-8d to have *syn* configuration, respectively. These results were further supported by the *J*-values and the calculated dihedral angles between H-7c and H-8c (J=~0 Hz, +71°), between H-8c and H-8b (J=7.0 Hz, −32°), and between H-8b and H-7b (J=11.0 Hz, +29°).

Finally, the stereochemistry between H-7d and H-8d was determined on the basis of the biogenesis along with NOEs mentioned above. (+)-Viniferol A (**1**) seems to be biogenetically synthesized from (+)-ϵ-viniferin (**2**) by way of an intermediate [I], as shown in Fig. 3. The route A gives (+)-viniferol A (**1**), and the route B gives (+)-hopeaphenol (**4**) and/or (−)-isohopeaphenol (**5**). The stereochemistry between H-7d and H-8d should, therefore, be *trans*, as is the stereochemistry between H-7a and H-8a of (+)-ϵ-viniferin (**2**). This consideration was also supported by the molecular mechanics calculations that revealed this configuration seemed to be the most stable. Consequently, the structure of (+)-viniferol A should be characterized as **1**.

3. Experimental

3.1. General

UV and IR spectra were recorded on JASCO Ubest V-560 (cell length 10 mm) and FT/IR-410 spectrometers, respectively. Optical rotations were measured with a JASCO P-1020 polarimeter (cell length 100 mm). ^1H and ^{13}C NMR spectra were recorded on JEOL ALPHA-600 (^1H: 600 MHz and ^{13}C: 150 MHz). Chemical shifts for ^1H and ^{13}C NMR are given in parts per million (δ) relative to solvent signal (methanol-d_4: δ_H 3.30 and δ_C 49.0, pyridine-d_5: δ_H 8.71 and δ_C 149.9) as internal standards, respectively. LR and HR FAB-MS were obtained with JEOL JMS HX-110 using *m*-nitrobenzylalcohol as matrix. Analytical TLC was performed on silica gel 60 F254 (Merck). Column chromatography was carried out on silica gel BW-820MH (Fuji Silysia Chemicals, Co. Ltd).

3.2. Isolation of viniferol A (1)

Stem of *V. vinifera* 'Kyohou' (17 kg) cultivated in Wakayama Prefecture was extracted with MeOH at room temperature to yield the extract (672 g). The methanol extract was dissolved in 50% aqueous MeOH (1.6 L) and extracted with *n*-hexane (1 L×3), chloroform (1 L×3), ethyl acetate (1 L×3), and *n*-butanol (1 L×3) to give the *n*-hexane (20.7 g), chloroform (21.1 g), ethyl acetate (60.4 g) and *n*-butanol solubles (154.8 g), respectively. A part of the

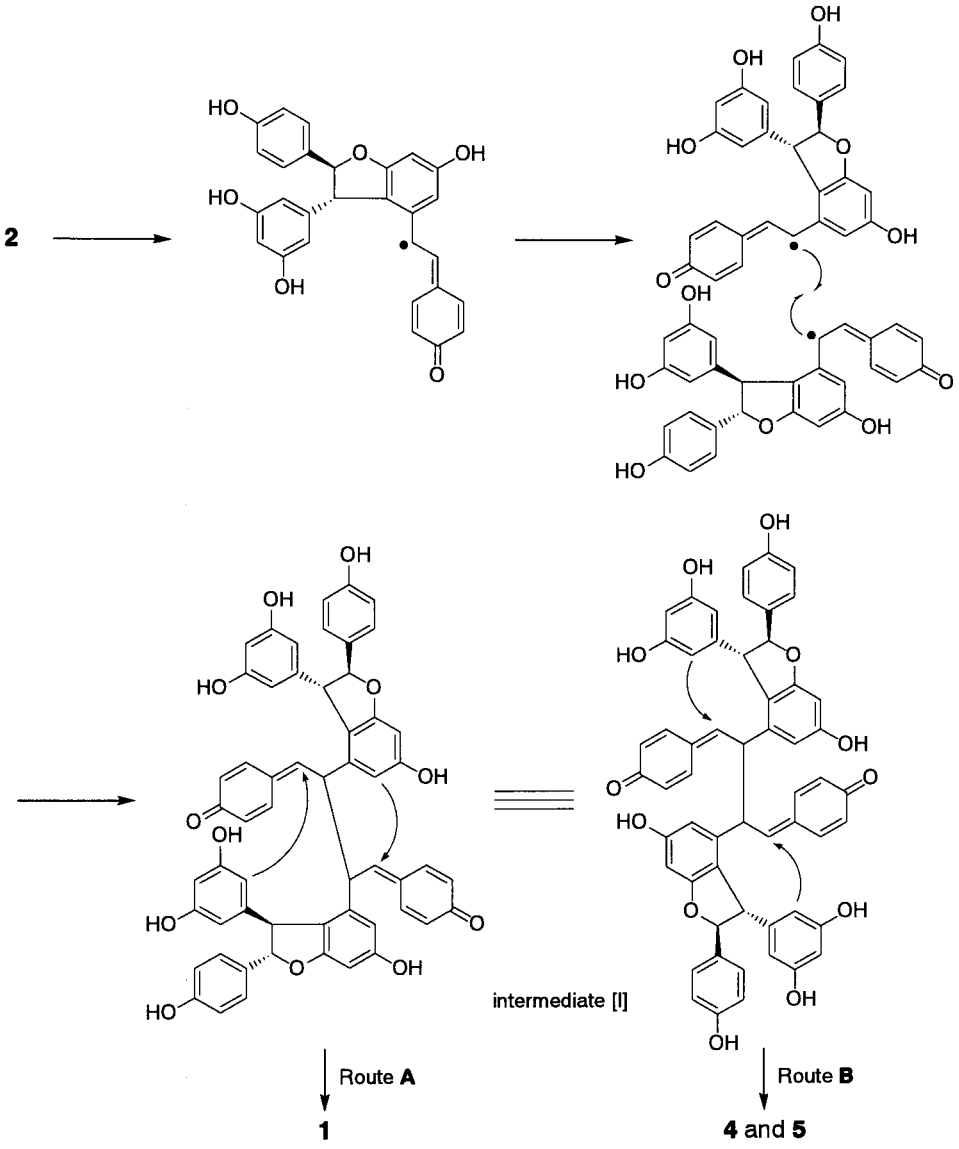

Figure 3. A plausible biogenetic pathway of (+)-viniferol A (**1**), (+)-hopeaphenol (**4**) and (−)-isohopeaphenol (**5**).

ethyl acetate fraction (29.5 g) was subjected to MPCC (45×450 mm) over silica gel (265 g) using a gradient solvent system of chloroform and methanol (20:1 to 0:1) to give 13 fractions (F-1 to F-13). F-11 (4.1 g, chloroform–methanol=4:1) was chromatographed (35×400 mm) over silica gel (110 g) eluting with increasing polarity of chloroform–methanol mixtures to give six fractions (F-111 to F-116). A reversed-phase MPCC (Develosil Lop C8-45S (45×450 mm), Nomura Chemical Co. Ltd.) of F-112 (785 mg) using a mixed solvent of methanol–water (55:45) followed by recycled HPLC (YMC-Pack C8-5 (20×250 mm), YMC Co. Ltd.) using the same solvent system afforded (+)-viniferol A (7.4 mg).

3.2.1. (+)-Viniferol A (1). $[\alpha]_D$=+136.6° (*c* 0.57, MeOH); a colorless amorphous solid; UV λ_{max} (MeOH) (nm (log ϵ)) 284 (3.99), 227 (sh, 4.80); IR ν_{max} (KBr) 3310, 1606, 1509, 1450 cm^{-1}; ^1H NMR and ^{13}C NMR data are shown in Tables 1 and 2; HRFAB-MS: *m/z* 907.2766 $[M+H]^+$ (907.2755 calculated for $C_{56}H_{43}O_{12}$).

3.3. Extraction with acetone and isolation of known resveratrol oligomers

The stem after extraction with methanol was further extracted with acetone at room temperature. After concentration under reduced pressure, the acetone solution gave an extract (229 g). The extract was fractionated by silica-gel column chromatography eluted stepwise using chloroform–methanol (9:1, 6:1, 4:1 and 3:1). An eluate with chloroform–methanol (6:1) was further fractionated by the repeated chromatography using silica-gel, reverse-phase MPCC and preparative TLC to give (+)-ε-viniferin,[2] (+)-vitisin A,[3] (+)-ampelopsin A[4] and (−)-malibatol A.[8] Moreover, an eluate with chloroform–methanol (4:1) afforded (+)-vitisin A, (+)-ampelopsin A[4] and (+)-ampelopsin F, and from a chloroform–methanol (3:1) fraction, (+)-ampelopsin A,[4] (+)-hopeaphenol,[6,7] (−)-isohopeaphenol,[7] (+)-resveratrol 10-*C*-glucoside[9] and (−)-resveratrol 11-*O*-glucoside[10] were obtained by the similar fractionation for the chloroform–methanol (6:1) fraction.

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11. The ^{1}H NMR spectrum in pyridine-d_5 showed the corresponding signals at (7.25 and 6.99 (each 2H, d, J=8.4 Hz), 6.99 and 6.85 (each 2H, d, J=8.4 Hz), 7.40 and 6.85 (each 2H, d, J=8.4 Hz), and 7.45 and 6.99 (each 2H, d, J=9.1 Hz).
12. In the cases of *syn*-configuration between H-7c and H-8a, such an upfield shift was not observed (balanocarpol (δ 5.14)[13] and isohopeaphenol (δ 5.37)[8]).
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14. Irradiation of H-2c (**6c**) showed a NOE at H-8a (4.1%) by the difference NOE experiment.