A NEW HYDROXYSTILBENE TETRAMER NAMED ISOHOPEAPHENOL FROM *VITIS VINIFERA* 'KYOHOU'

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Abstract —— A new hydroxystilbene tetramer named isohopeaphenol was isolated from the cork of *Vitis vinifera* 'Kyohou' and its structure was elucidated on the basis of spectroscopic evidence.

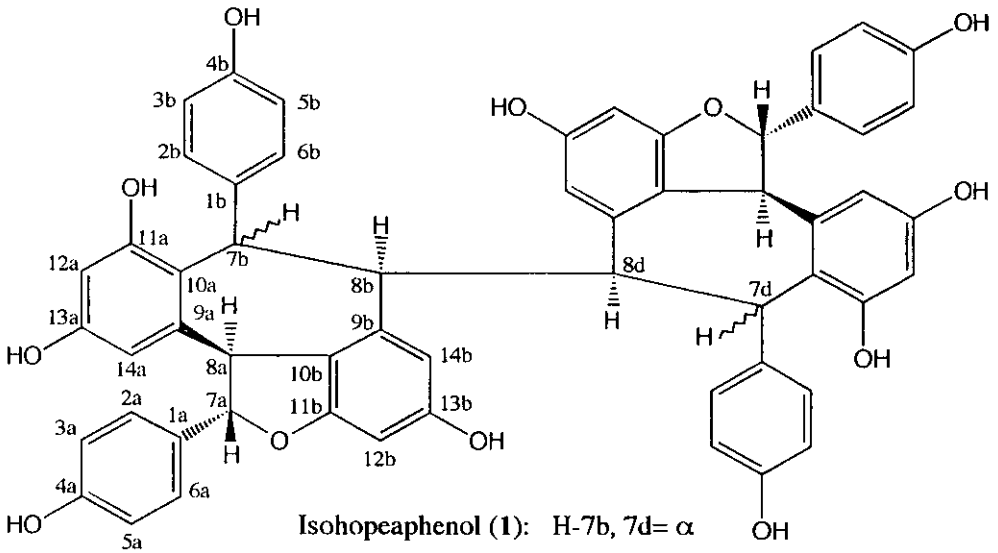
In our previous papers,[1,2] we reported the isolation and structures of hydroxystilbene oligomers and lupane-type triterpenes from the cork of *Vitis vinifera* 'Kyohou' cultivated in Wakayama Prefecture. Our continuing investigation of the constituents of the above plant led to the isolation of a new hydroxystilbene tetramer named (-)-isohopeaphenol (**1**) together with (+)-hopeaphenol (**2**). In this paper we describe the isolation and structure of isohopeaphenol (**1**).

Isolation The residue extracted by methanol described in the previous paper[2] was further extracted by acetone. The obtained acetone extract was partitioned between hexane, chloroform and ethyl acetate, and water to give the corresponding solubles, respectively. The ethyl acetate soluble fraction was repeatedly subjected to medium-pressure column chromatography (MPCC) on silica gel, and then to high-performance liquid chromatography (HPLC) on reversed-phase silica gel to give a mixture of (-)-isohopeaphenol (**1**) and (+)-hopeaphenol (**2**). The mixture was finally subjected to recycled HPLC on reversed-phase silica gel to give (-)-isohopeaphenol (**1**) and (+)-hopeaphenol (**2**) in 0.29 % and 0.52 % yields, respectively, on the basis of the extract. The relative structure of (+)-hopeaphenol (**2**) was identified by comparison with an authentic sample.[3] The reported hopeaphenol isolated from the Dipterocarpaceae,[4] Cyperaceae,[3] and Leguminosae[5] plants, respectively, has (-)-optical rotation and the absolute configuration of (-)-hopeaphenol was already established by the X-Ray crystal structure analysis.[6] Our hopeaphenol is its antipode and the absolute configuration is shown as **2**. This is the first isolation of (+)-hopeaphenol.

Structure of (-)-Isohopeaphenol (-)-Isohopeaphenol (**1**), $[\alpha]_D$ -114.5° (*c* 0.44, MeOH), was found to have the molecular formula $C_{56}H_{42}O_{12}$ determined by the high resolution FABMS, which corresponded to a tetramer of 3,5,4'-trihydroxystilbene (resveratrol). However, both ^1H and ^{13}C NMR

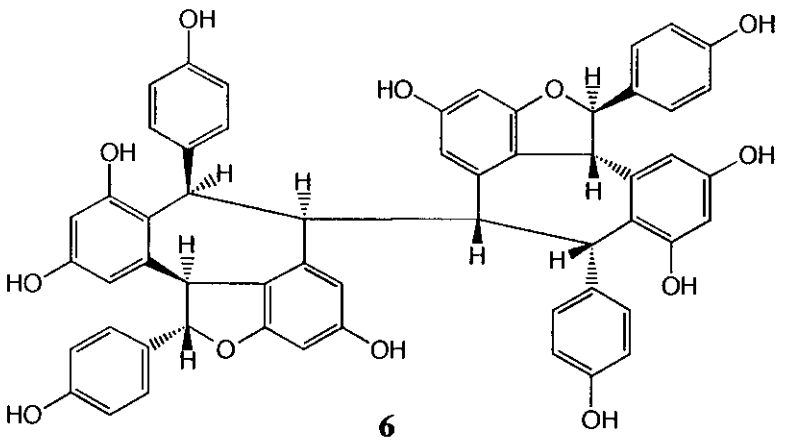
Table 1. ^{1}H and ^{13}C NMR Data of Isohopeaphenol (**1**) and Hopeaphenol (**2**)

	Isohopeaphenol (**1**)		Hopeaphenol (**2**)	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1a		133.77 (s)		131.02 (s)
2a	7.52 (d, 8.8)	131.15 (d)	7.09 (d, 8.8)	130.51 (d)
3a	6.98 (d, 8.8)	116.88 (d)	6.72 (d, 8.8)	116.18 (d)
4a		159.10 (s)		158.81 (s)
5a	6.98 (d, 8.8)	116.88 (d)	6.72 (d, 8.8)	116.18 (d)
6a	7.52 (d, 8.8)	131.15 (d)	7.09 (d, 8.8)	130.51 (d)
7a	5.56 (d, 10.3)	94.59 (d)	5.80 (d, 12.5)	88.97 (d)
8a	5.37 (d, 10.3)	54.18 (d)	4.12 (d, 12.5)	49.94 (d)
9a		141.08 (s)		142.50 (s)
10a		118.84 (s)		122.01 (s)
11a		160.63 (s)		159.40 (s)
12a	6.32 (d, 2.2)	102.66 (d)	6.36 (d, 2.2)	100.99 (d)
13a		158.25 (s)		157.23 (s)
14a	6.19 (d, 2.2)	107.40 (d)	6.21 (d, 2.2)	106.25 (d)
1b		138.19 (s)		136.02 (s)
2b	6.33 (d, 8.8)	130.10 (d)	6.89 (d, 8.8)	129.72 (d)
3b	6.30 (d, 8.8)	114.93 (d)	6.54 (d, 8.8)	115.32 (d)
4b		155.12 (s)		155.47 (s)
5b	6.30 (d, 8.8)	114.93 (d)	6.54 (d, 8.8)	115.32 (d)
6b	6.33 (d, 8.8)	130.10 (d)	6.89 (d, 8.8)	129.72 (d)
7b	5.03 (d, 1.5)	44.08 (d)	5.75 (brs)	41.54 (d)
8b	3.36 (t, 1.5)	53.37 (d)	3.85 (brs)	48.57 (d)
9b		142.25 (s)		141.23 (s)
10b		117.72 (s)		119.55 (s)
11b		159.15 (s)		159.28 (s)
12b	5.79 (d, 2.2)	95.33 (d)	5.73 (d, 2.2)	95.28 (d)
13b		156.92 (s)		156.98 (s)
14b	5.42 (d, 2.2)	110.19 (d)	5.07 (d, 2.2)	111.77 (d)



Isohopeaphenol (**1**): H-7b, 7d= α

(+)-Hopeaphenol (**2**): H-7b, 7d= β



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spectra of isohopeaphenol (**1**) showed half the signals corresponding to the molecular formula. This indicated that isohopeaphenol was comprised of two equivalent units. The ^{1}H NMR spectrum exhibited signals for eight sets of *ortho*-coupled aromatic hydrogens (δ 7.52, 6.98 (each 4H, d, *J*= 8.8 Hz); 6.33, 6.30 (each 4H, d, *J*= 8.8 Hz) and four sets of *meta*-coupled aromatic hydrogens (δ 6.32, 6.19 (each 2H, d, *J*= 2.2 Hz); 5.79, 5.42 (each 2H, d, *J*= 2.2 Hz)). The aliphatic hydrogen signals at δ 5.56 and 5.37 (each 2H, d, *J*= 10.3 Hz) suggested the presence of two dihydrobenzofuran moieties bearing 4-oxyphenyl and 3,5-dioxyphenyl groups characteristic of oligostilbenes biosynthesized from resveratrol molecules. The other coupled signals at δ 5.03 and 3.36 (each 2H, d, *J*= 1.5 Hz), due to the hydrogens attached to the carbons at δ 44.08 (C-7b, 7d) and 53.37 (C-8b, 8d) suggested the presence of vicinal methines. The latter signal was especially assigned to the protons on the positions of dimerization between the two ampelopsin B (**3**) moieties.[7,8] These facts indicated isohopeaphenol to be an isomer of hopeaphenol.[3-5] However, the chemical shift value (δ 5.37) of H-8a (8c) of **1** is markedly different from that (δ 4.12) of **2**. These

phenomena were observed in the case of balanocarpol (**4**)[9] ($\delta_{H\text{-}8a}$ 5.14), and ampelopsin B (**3**)[7] ($\delta_{H\text{-}8a}$ 4.19) and ampelopsin A (**5**)[7] ($\delta_{H\text{-}8a}$ 4.02). In the cases of **2**, **3** and **5**, the high-field shift of H-8a is due to the anisotropic effect of the hydroxyphenyl group on C-7b. This is observed only when the hydroxyphenyl group is situated *cis* to H-8a.[10] In DIF-NOE experiments of **1**, the NOEs between H-8a and H-8b (4.1%), and H-7b and H-8b (3.7%) were observed, respectively. These observations together with the coupling constant value (J= 10.3 Hz) between H-7a and H-8a indicated the relative stereochemistry of **1** to be *trans* between H-7a and H-8a, *cis* between H-8a and H-7b, and *cis* between H-8a and H-8b. Finally, the relative stereochemistry between C-8b and C-8d is discussed as follows. Isohopeaphenol (**1**) should be represented as **1** or **6** except for the absolute configuration. The structure **1** has no symmetrical plane as seen in the case of a biflavonoid, chamaejasmine (**7**) ($[\alpha]_D$ -61.2°), while the structure **6** has a symmetrical plane as seen in the case of isochamaejamin (**8**) ($[\alpha]_D$ 0°).[11,12] Therefore, isohopeaphenol (**1**) ($[\alpha]_D$ -114.5°) has the same stereochemistries as those of hopeaphenol (**2**) ($[\alpha]_D$ +374.8°) as shown above.[3,4,6] However, the absolute configuration of isohopeaphenol (**1**) still remains to be clarified.

EXPERIMENTAL

Optical rotations were recorded on a JASCO DIP-181 digital polarimeter using a 100 mm length quartz cell at 25 °C. UV spectrum was taken in CH_3OH with a JASCO UVIDEC-610 spectrophotometer. IR spectra were taken on a JASCO FT/IR-5000 spectrophotometer. MS spectra were obtained under FAB conditions with a JEOL HX-110 spectrometer. ^{1}H NMR, ^{13}C NMR, two-dimensional (2D) NMR and difference NOE spectra were measured with JEOL α-400 and α-600 spectrometers.

Isolation The residue extracted by methanol described in the previous paper[2] was extracted by acetone (30 L x 2,) at rt to give an extract (83.3 g). A part of the acetone extract (41.7 g) was partitioned between hexane, chloroform and ethyl acetate, and water to give hexane (5.8 g), chloroform (9.6 g) and ethyl acetate (15.9 g) solubles, respectively. The ethyl acetate soluble fraction (15.9 g) was subjected to MPCC over silica gel (Fuji Silysia Chemical Ltd., BW-820MH, 270 g) using a mixture of chloroform and methanol [20 : 1 (1450 mL), 10 : 1 (990 mL), 5 : 1 (1080 mL)], acetone (500 mL) and then methanol (500 mL) to give 5 fractions (0.64 g, 1.96g, 5.40 g, 4.15 g, 0.32 g, respectively). A part of the fourth fraction eluted by acetone (1.05 g) was to MPCC over silica gel (Katayama Chemical Ltd., silica gel 60K 230, 73 g) using a gradient solvent system of chloroform and methanol [4 : 1 (1000 mL), 1 : 1 (600 mL), 1 : 2 (600 mL)] to give three fractions (16 mg, 130 mg, 146 mg, respectively). The second fraction eluted by a mixture of chloroform and methanol (1 : 1) (130 mg) was subjected to recycled HPLC (YMC-Pack C8-5, ϕ 20 x 250 mm) using a mixture of methanol and water (60 : 40) to give hopeaphenol (**2**) (55 mg) and isohopeaphenol (**1**) (31 mg).

Isohopeaphenol (**1**)

Pale brownish powder. $[\alpha]_D$ -114.5° (*c* 0.44, MeOH). HR-FABMS, *m/z* 907.2818 $[M+H]^+$ ($C_{56}H_{42}O_{12}$ requires: 907.2754). UV (MeOH) λ 284 (ε 16800), 229 (ε 66600), 210 nm (ε 98800). CD (MeOH) Δε (nm); -3.4 (290), +24 (240), -62 (213). IR ν_{max} (KBr) 3300 br, 1615 cm^{-1}. ^1H NMR and ^{13}C NMR given in Table 1.

Hopeaphenol (**2**)

Pale brownish powder. $[\alpha]_D$+374.8 ° (*c* 0.78, MeOH). HR-FABMS, *m/z* 907.2810 $[M+H]^+$ ($C_{56}H_{42}O_{12}$ requires: 907.2754). UV (MeOH) λ 283 (ε 14900), 228 (ε 69100), 210 nm (ε 98800). CD (MeOH) Δε (nm); +19 (286), +55 (230), +80 (211). IR ν_{max} (KBr) 3300 br, 1600 cm^{-1}. ^1H NMR and ^{13}C NMR given in Table 1.

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