

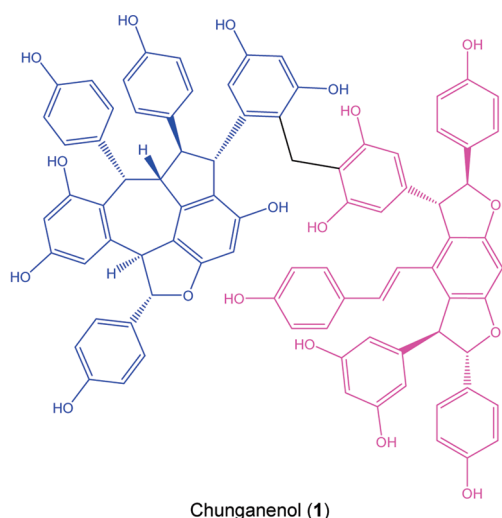
Chunganenol: An Unusual Antioxidative Resveratrol Hexamer from *Vitis chunganensis*

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Received June 25, 2009



Chunganenol (**1**), an unusual resveratrol hexamer, has been isolated from *Vitis chunganensis*. Its unprecedented structure has been assigned through detailed analyses of 2D NMR data and consideration of oligostilbene biosynthetic pathway, representing the first highly condensed stilbene oligomer from Vitaceae plants. It also featured a novel interunit linkage where two stilbene units are connected by a methylene bridge. Moreover, **1** exhibited potent and selective quenching effects on singlet oxygen, and might play a role in the antioxidative defense system of the plant.

Plants of *Vitis* (Vitaceae family), commonly known as the genus of grape, are rich sources of stilbenoids, especially resveratrol (3,5,4'-trihydroxystilbene) oligomers,<sup>1</sup> which have attracted considerable attention owing to their structural complexity and various important biological activ-

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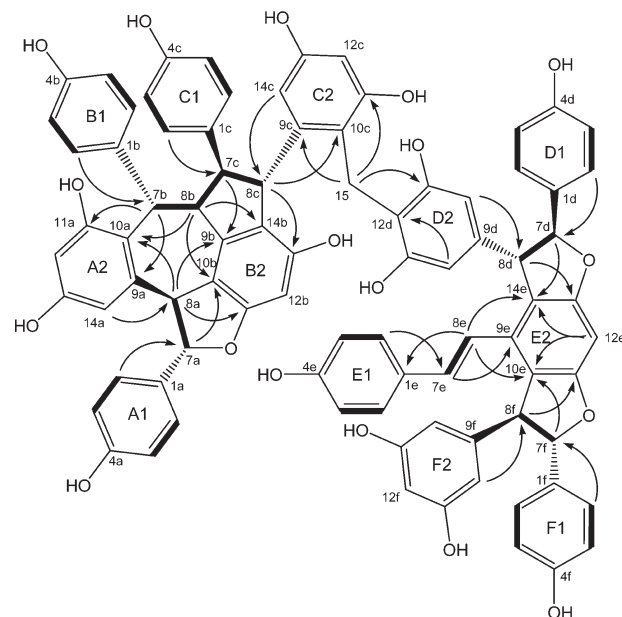


FIGURE 1. Key HMBC (indicated by arrows from <sup>1</sup>H to <sup>13</sup>C) and <sup>1</sup>H–<sup>1</sup>H COSY correlations (indicated by bold lines) for **1**.

ities.<sup>2</sup> As naturally occurring polyphenols, resveratrol oligomers possess antioxidant activity,<sup>2</sup> including potent scavenging effects against reactive oxygen species (ROS).<sup>3</sup> In our recent report, resveratrol oligomers from *Parthenocissus laetevirens* (Vitaceae family) have shown strong antioxidant activities, thus, they have been hypothesized to play a role in the antioxidative defense system of the plant.<sup>4</sup>

*Vitis chunganensis* Hu is a species of wild grapes distributed in southeast China and is traditionally used as folk medicine for the treatment of infectious hepatitis and physical injury.<sup>5</sup> Recently, we have developed a methodology for the preparative isolation and purification of three antioxidative resveratrol oligomers, namely amurensin G (**5**),<sup>6</sup> vitisin A (**6**),<sup>7</sup> and hopeaphenol (**7**),<sup>8</sup> from a crude sample of *V. chunganensis* through a one-step separation by high-speed counter-current chromatography,<sup>9</sup> which represented the first study on this species. The following phytochemical investigation on this species led to the isolation of chunganenol (**1**), an unusual resveratrol hexamer, along with seven known stilbenoids. Their antioxidant activities

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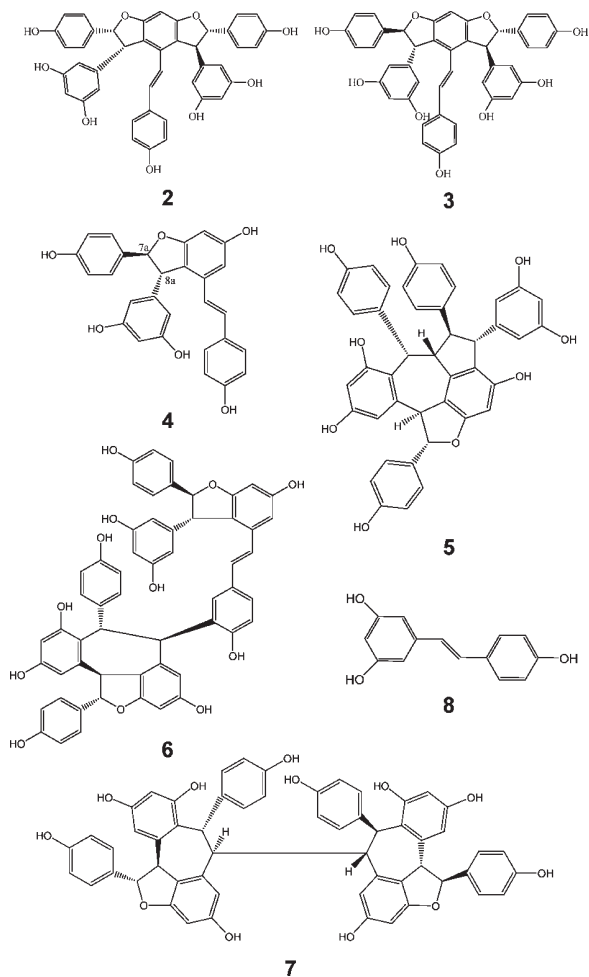
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were evaluated by DPPH assay, and the scavenging effects of **1** against ROS were investigated by EPR spin-trapping technique.

Whole plants of *V. chunganensis* were extracted with methanol at room temperature to yield a crude extract. This crude extract was then partitioned between ethyl acetate and water. The ethyl acetate solubles were separated by silica gel column chromatography (CC), followed by reversed-phase C-18 CC and preparative HPLC to afford compounds **1**–**8**.



Chunganenol (**1**), obtained as a colorless amorphous powder, was determined to have a molecular formula of  $C_{85}H_{64}O_{18}$  from its HR-ESI-MS, which corresponded to a resveratrol hexamer plus one C. Analysis of the 1D and 2D NMR data (Figure 1) showed the presence of two trimeric units (trimeric unit I: resveratrol units A–C; trimeric unit II: resveratrol units D–F) matching the co-occurring resveratrol trimer **5** and **3**, respectively. Key HMBC correlations (Figure 1) observed between H-15/C-9c, C-11c, and C-11d(13d) demonstrated that the aromatic rings C2 and D2 were connected via a methylene group (C-15) at C-10c and C-12d, which was supported by NOE correlation between H-15 $\alpha$  and H-8c (Figure 2). Therefore, trimeric units I and II were linked by a methylene bridge, which represented an unprecedented stilbene–interunit linkage. As a result, the planar structure of **1** was established as shown in Figure 1,

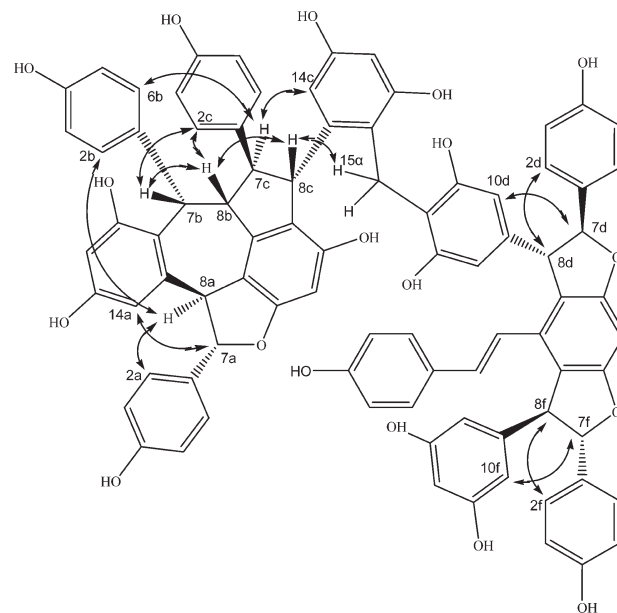


FIGURE 2. Key NOE correlations for **1**.

while detailed analysis of the 2D NMR spectra allowed the assignment of all proton and carbon signals as shown in Table 1.

The relationship between H-7e and H-8e was found to be *trans* on the basis of the *J* value (16.8 Hz).<sup>10</sup> Relative configurations of the chiral centers were determined by analysis of the NOESY spectrum as shown in Figure 2. The relative stereochemistry was found to be identical with that of amurensin G (**5**)<sup>6</sup> in trimeric unit I. In the other trimeric unit II, the relative configurations were assumed found to be those of gnetin H (**3**),<sup>11</sup> because this co-occurring compound **3** was identified as (+)-gnetin H, rather than ampelopsin E<sup>12</sup> (see Figure S1 in the Supporting Information), which was optically inactive. Many reports have suggested that most naturally occurring oligostilbenes possessing at least one five-membered oxa-cyclic ring were formed from resveratrol via the dimer  $\epsilon$ -viniferin (**4**), which has been considered as a main biogenetic route for oligostilbenes.<sup>10a,13</sup> Since only (+)- $\epsilon$ -viniferin (7aS,8aS)<sup>14</sup> was isolated from this plant, the absolute configuration of **1** was proposed to be (7aS,8aS,7bS,8bS,7cR,8cR,7dS,8dS,7fS,8fS) as shown, considering the biosynthesis of oligostilbenes. However, this assignment awaits future experimental verification.

The highly condensed stilbene oligomer (HCSO), which was composed of more than five stilbene monomers, is unusual in nature. Hitherto, there have only been five HCSOs discovered from the plant kingdom, namely vaticanol D,<sup>15a</sup> vaticanols H–J,<sup>15b</sup> and vateriaphenol A,<sup>15c</sup> all of which have

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TABLE 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data of Chunganenol (1) in Acetone- $d_6$ <sup>a</sup>

position	$\delta_{\text{H}}$ (mult., J Hz)	$\delta_{\text{C}}$	position	$\delta_{\text{H}}$ (mult., J Hz)	$\delta_{\text{C}}$
1a		130.8	1d		133.9
2a (6a)	7.28 (2H, d, 8.6)	130.2	2d (6d)	7.16 (2H, d, 8.6)	127.9 <sup>j</sup>
3a (5a)	6.79 (2H) <sup>b</sup>	115.9	3d (5d)	6.81 (2H) <sup>b</sup>	116.1 <sup>h</sup>
4a		158.6	4d		158.1 <sup>k</sup>
7a	5.87 (1H, d, 11.8)	90.6	7d	5.38 (1H, d, 5.1)	94.0
8a	4.50 (1H, d, 11.9)	48.9	8d	4.45 (1H, d, 5.0)	57.8
9a		141.7	9d		144.4
10a		124.8	10d (14d)	6.34 (2H, s)	107.7
11a		155.9 <sup>f</sup>	11d (13d)		156.7
12a	6.38 (1H, d, 2.2)	101.7	12d		113.7
13a		156.5	1e		130.0
14a	6.21 (1H, d, 1.7)	105.9	2e (6e)	6.88 (2H, d, 8.6)	128.5
1b		133.2 <sup>g</sup>	3e (5e)	6.61 (2H, d, 8.6) <sup>e</sup>	116.1 <sup>h</sup>
2b (6b)	7.19 (2H, d, 8.6)	130.5	4e		158.1 <sup>k</sup>
3b (5b)	6.71 (2H, d, 8.5)	115.5	7e	6.61 (1H) <sup>c</sup>	133.8 <sup>l</sup>
4b		155.9 <sup>f</sup>	8e	6.51 (1H, d, 16.8)	122.2
7b	5.27 (1H, d, 3.0)	37.5	9e		133.2 <sup>g</sup>
8b	3.78 (1H) <sup>c</sup>	51.9	10e		119.8
9b		144.1	11e		162.60
10b		116.3	12e	6.43 (1H, s)	91.2
11b		159.2	13e		162.62
12b	6.07 (1H, s)	96.5	14e		119.7
13b		153.8	1f		133.8 <sup>l</sup>
14b		121.8	2f (6f)	7.24 (2H, d, 8.6)	127.9 <sup>j</sup>
1c		132.0	3f (5f)	6.84 (2H, d, 8.6)	116.1 <sup>h</sup>
2c (6c)	7.01 (2H, d, 8.5)	129.8	4f		158.2
3c (5c)	6.69 (2H, d, 8.5)	116.1 <sup>h</sup>	7f	5.43 (1H, d, 5.2)	94.1
4c		156.9 <sup>f</sup>	8f	4.55 (1H, d, 5.2)	57.9
7c	3.75 (1H) <sup>c</sup>	63.6	9f		147.1
8c	5.39 (1H, d, 10.2)	52.1	10f (14f)	6.25 (2H, d, 2.2) <sup>d</sup>	106.8
9c		144.2	11f (13f)		159.8
10c		118.0	12f	6.18 (1H, t, 2.1)	102.2
11c		156.9 <sup>f</sup>	15 $\alpha$	3.57 (1H, d, 15.2)	19.7
12c	6.24 (1H) <sup>d</sup>	102.3	15 $\beta$	3.21 (1H, d, 15.2)	
13c		157.6			
14c	6.46 (1H, d, 2.5)	107.1			

<sup>a</sup> $^1\text{H}$  NMR spectra were measured at 500 MHz, and  $^{13}\text{C}$  NMR spectra were run at 125 MHz. <sup>b–f</sup>Overlapping signals.

been isolated from Dipterocarpaceous plants. Therefore, chunganenol (1) is the sixth naturally occurring HCSO discovered, and is the first one from the family of Vitaceous plants.

Since the structures of trimeric units I and II in 1 were very similar to those of 5 and 3, respectively, 1 was probably biosynthesized from the condensation of these two trimers. However, the biosynthetic origin of the methylene bridge is still unclear.

The stilbenoids (1–8) from *V. chunganensis* were first screened for their antioxidant activities by DPPH radical assay, which has been widely used for the evaluation of antioxidant activities of natural products.<sup>16</sup> The results are summarized in Table 2. Compound 1 showed potent antioxidant activity, which was comparable with that of vitamin E. Interestingly, the antioxidant activity of this hexamer was

TABLE 2. Antioxidant Activities of the Stilbene Oligomers (1–8) from *V. chunganensis*

compds	DPPH radical IC <sub>50</sub> ( $\mu\text{M}$ ) <sup>a</sup>
1	37.3 $\pm$ 0.8
2	188 $\pm$ 4
3	251 $\pm$ 6
4	62.2 $\pm$ 3
5	138 $\pm$ 4 <sup>b</sup>
6	42.4 $\pm$ 1 <sup>b</sup>
7	115 $\pm$ 4 <sup>b</sup>
8	73.2 $\pm$ 2 <sup>b</sup>
vitamin E	33.6 $\pm$ 1

<sup>a</sup>IC<sub>50</sub> values were expressed as means  $\pm$  standard deviation. <sup>b</sup>Results published elsewhere<sup>9</sup> were listed here for comparison.

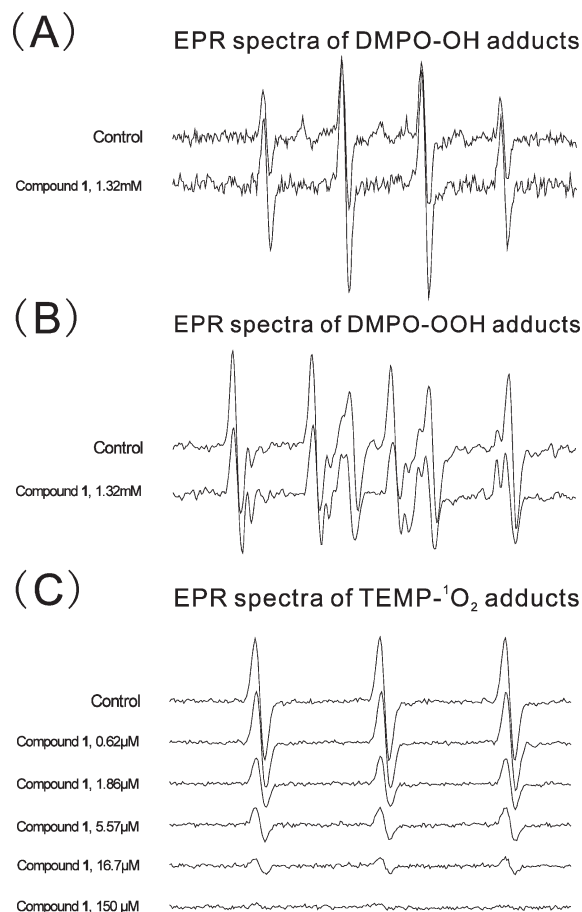


FIGURE 3. Scavenging and quenching effects of 1 on ROS, including (A) hydroxyl radical  $\cdot\text{OH}$ , (B) superoxide anion  $\text{O}_2^{\cdot-}$ , and (C) singlet oxygen  $^1\text{O}_2$  as determined by EPR spin-trapping technique.

much stronger than that of its precursive trimers (3 and 5). Therefore, the condensation yielding 1 was probably an antioxidant activity-promoting reaction. In our previous paper, we have proposed a new mechanism for the antioxidative defense system in *P. laetevirens*, which enhances antioxidant capacity in response to photooxidative stress.<sup>4</sup> In the present case, the biosynthesis of 1 might have also played a role in the antioxidative defense system of *V. chunganensis*, which could reinforce antioxidant activity to counteract other environmental stresses.

In recent decades, ROS have been considered as important causative agents of various human diseases including cancer, heart diseases, Parkinson's disease, autoimmune disease,

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and stroke.<sup>17</sup> Therefore, **1** was investigated further for its scavenging activity against ROS through EPR spin-trapping technique, which provides more reliable and specific results.<sup>18</sup> The EPR data (Figure 3) demonstrated that **1** was a potent singlet oxygen quencher (Figure 3C), but not an effective scavenger of hydroxyl radical and superoxide anion (Figure 3A,B). The concentration of **1** required to inhibit 50% of singlet oxygen generation was found to be 1.42  $\mu\text{M}$ , which was much stronger than that of epigallocatechin gallate (EGCG,  $\text{IC}_{50} = 14.5 \mu\text{M}$ ). Therefore, **1** could be considered a selective singlet oxygen quencher, which may have potential pharmacological applications in singlet oxygen-mediated diseases, including erythropoietic protoporphyria, pellagra, and cataractogenesis.

### Experimental Section

**Plant Material.** The whole plants of *V. chunganensis* were collected in May 2007 in Jinhua, Zhejiang Province, China. The material was identified by Dr. Yunpeng Zhao (College of Life Sciences, Zhejiang University, Hangzhou, China). A voucher specimen (No. P070511) was deposited at the Department of Biology, Zhejiang University, China.

**Extraction and Isolation.** The plant material (3.5 kg) was extracted three times with MeOH ( $3 \times 20 \text{ L}$ ) at room temperature. The solvent was evaporated in vacuo to produce a concentrated MeOH extract (309 g), which was then diluted with  $\text{H}_2\text{O}$  (1.5 L) to give an aqueous solution (1.5 L). The aqueous solution was extracted with EtOAc three times ( $3 \times 3.0 \text{ L}$ ). The combined EtOAc layers were concentrated to dryness in vacuo to provide an EtOAc extract (175 g), which was then subjected to silica gel CC (1700 g, 7 cm diameter) eluted with light petroleum–EtOAc mixtures (50:1 to 1:10) to yield 10 fractions. Fraction 5 (3.8 g) was subjected to preparative HPLC (column Shimadzu Shimpack-C18,  $250 \times 20 \text{ mm i.d.}$ ; solvent MeOH– $\text{H}_2\text{O}$ , 50%:50%; flow rate 8 mL/min; detection 280 nm) to afford three pure isolates **1** ( $t_{\text{R}} = 93 \text{ min}$ , 30 mg),

**2** ( $t_{\text{R}} = 76 \text{ min}$ , 850 mg), and **3** ( $t_{\text{R}} = 121 \text{ min}$ , 460 mg). Fraction 6 (10.4 g) was separated by preparative HPLC with similar condition except the ratio of MeOH: $\text{H}_2\text{O}$  (40%:60%) to give compounds **4** ( $t_{\text{R}} = 63 \text{ min}$ , 540 mg), **5** ( $t_{\text{R}} = 89 \text{ min}$ , 920 mg), and **6** ( $t_{\text{R}} = 144 \text{ min}$ , 670 mg). Fraction 7 (5.8 g) was separated by RP-18 CC (MeOH– $\text{H}_2\text{O}$ , 35%:65%) to yield compounds **7** (310 mg) and **8** (170 mg).

**Chunganenol (1):** colorless amorphous powder;  $[\alpha]_{\text{D}}^{20} +54.1$  ( $c$  0.45, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 226 (4.4), 283 (3.7), 327 (3.7) nm; IR (KBr)  $\nu_{\text{max}}$  3384, 1612, 1514, 1448, 1337, 1242, 1172, 1076, 1008, 833  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; HR-ESI-MS  $m/z$   $[\text{M} - \text{H}]^-$  1371.3964 (calcd for  $\text{C}_{85}\text{H}_{63}\text{O}_{18}$ , 1371.4020).

Compounds **2–8** were determined as (+)-amurensin B (**2**,  $[\alpha]_{\text{D}}^{20} +95$  ( $c$  0.18, MeOH)),<sup>10b</sup> (+)-gnetin H (**3**,  $[\alpha]_{\text{D}}^{20} +9$  ( $c$  0.19, MeOH)),<sup>11</sup> (+)- $\epsilon$ -viniferin (**4**,  $[\alpha]_{\text{D}}^{20} +32$  ( $c$  0.27, MeOH)),<sup>14,19</sup> (+)-amurensin G (**5**,  $[\alpha]_{\text{D}}^{20} +22$  ( $c$  0.28, MeOH)),<sup>6</sup> (+)-vitisin A (**6**,  $[\alpha]_{\text{D}}^{20} +174$  ( $c$  0.11, MeOH)),<sup>7</sup> (+)-hopeaphenol (**7**,  $[\alpha]_{\text{D}}^{20} +330$  ( $c$  0.18, MeOH)),<sup>8</sup> and resveratrol (**8**)<sup>20</sup> by comparison of the spectroscopic data with those in the literature.

**Determination of Antioxidant Activities.** The antioxidant activities of all the compounds were first screened by DPPH assay as previously described.<sup>4</sup> Vitamin E was used as a positive control. Compound **1** was further investigated for its scavenging effects on reactive oxygen species (ROS, including hydroxyl radical  $\cdot\text{OH}$ , superoxide anion  $\text{O}_2^{\cdot-}$  and singlet oxygen  $^1\text{O}_2$ ) by using the EPR spin-trapping technique as previously described.<sup>3a</sup> Mannitol and EGCG were used as a positive control.

**Acknowledgment.** Financial support from the NSFC (20775069) and the NCET-06-520 is gratefully acknowledged.

**Supporting Information Available:** General Experimental Methods, 1D and 2D NMR spectra, as well as HRMS data of compound **1**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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