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Cytotoxic Phenylpropanoids from Carrot

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Carrot is widely used as a foodstuff. The active components such as β-carotene and panaxynol have been studied by many researchers. In this investigation of nonpolar active components from carrot, a new phenylpropanoid, epilaserine oxide (**3**), was isolated along with six known compounds, laserine (**1**), 2-epilaserine (**2**), panaxynol (**4**), ginsenoyne K (**5**), (8*E*)-1,8-heptadecadiene-4,6-diyne-3,10-diol (**6**), and vaginatin (**7**). Their structures were deduced on the basis of spectroscopic methods. Significant cytotoxicity of 2-epilaserine against HL-60 cells was observed, which implied that phenylpropanoids were cytotoxic compounds in carrot. Laserine and 2-epilaserine in carrots from diverse locations in China were quantified by HPLC.

KEYWORDS: ***Daucus carota*; phenylpropanoid; epilaserine oxide; 2-epilaserine; cytotoxic activity**

INTRODUCTION

Epidemiological investigations have indicated that a high daily intake of vegetables and fruits protects against certain types of cancer, cardiovascular diseases, and diabetes (*1*). With wide usage as a foodstuff, carrot has been investigated to evaluate the nutritional value and potential beneficial effects on human health. One of its components, β-carotene, has attracted the attention of many researchers for its multiple bioactivities in different fields (*2, 3*). Recently, other nonpolar secondary metabolites of carrot, especially polyacetylenes, have received more attention as well (*4*). Eugenin, 6-methoxymellein, gazarin, and three polyacetylenes have been reported from carrot, and panaxynol was proved to be the most intensely bitter-tasting compound of carrot (*5*). Results of a bioactivity assay implied that the negative correlation of low cancer risk with high intake of natural carotene was produced by the co-occurrence of carotenes and polyacetylenes (*6*).

Phenylpropanoids are common in the Umbelliferae family, and they have been reported to present a wide range of biological activities (*7*). In our investigation of nonpolar active components from carrot, three phenylpropanoids and one sesquiterpene were obtained for the first time along with three polyacetylenes (**Figure 1**). A bioactivity assay showed that phenylpropanoids were cytotoxic compounds in carrot.

MATERIALS AND METHODS

Plant Material. Carrots (red) were commercially obtained from markets in China.

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-400 and AM-500 spectrometers using $CDCl_3$ as solvent. The HRESI-MS experiment was performed using a Bruker APEXIII 7.0 TESLA FTMS spectrometer. Optical rotation was measured on a P1030 polarimeter.

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HPLC-VWD. Analytical HPLC was performed on an Agilent 1100 system equipped with a 250 × 4.6 mm i.d., 5 μm, Kromasil C-18 column and an autosampler and a variable wavelength detector (VWD): oven temperature, 25 °C; detection wavelength, 220 nm; injection volume, 5 μL; mobile phases, A (MeOH) and B (water); flow rate, 0.8 mL/min; linear gradient, 0 min, 80% A/20% B, 20 min, 80% A/20% B, 21 min, 100% A; stop time, 35 min. **1** (laserine) and **2** (2-epilaserine) were observed with retention times of 11.9 and 12.6 min, respectively. HPLC chromatograms for extracts of carrots from diverse locations are shown in **Figure 2**.

Extraction and Isolation. Fresh carrots (7 kg) were cut into small pieces and extracted with EtOH three times (12 L × 3). After removal of solvent, the residue was partitioned between Et_2O and water to give 6 g of Et_2O extract. The extract was subjected to silica gel column (40 × 6 cm) chromatography, eluting with petroleum ether (60–90 °C) containing increasing concentrations of EtOAc. Each fraction was 300 mL. Fractions 49–56 (petroleum ether /EtOAc 20:1) and 42–46 (petroleum ether/EtOAc 20:1) containing, respectively, **1**−**4** and **5**−**7** were isolated using a semipreparative RP-HPLC column (250 × 10 mm i.d., 10 μm, Kromasil from EKA Chemicals), which was performed on an Agilent 1100 system equipped with a VWD. The detection wavelength was 254 nm. The flow rate was 0.8 mL/min. The linear gradient mobile phase of fractions 42–46 was as follows: 0 min, 80% A (MeOH) and 20% B (water); 70 min, 90% A and 10% B; 80 min, 100% A. The mobile phase for fractions 49–56 was 80% A and 20% B.

Compound 1 (laserine): colorless oil; ESI-MS, *m*/*z* 405 $[M + H]^+$; $[\alpha]_D^{25}$ 3.4° (*c* 0.096 in $CHCl_3$); ^{1}H NMR, δ 6.58 (1H, br s; H-2′), 6.57 (1H, br s; H-6′), 6.09 (1H, qq, *J* = 5.6, 1.3 Hz; H-3″, 6.05 (1H, qq, *J* = 5.6, 1.3 Hz; H-3‴, 5.96 (2H, s; H-7′), 5.77 (1H, d, *J* = 7.3 Hz; H-1), 5.35 (1H, pent, *J* = 6.5 Hz; H-2), 3.89 (3H, s; OMe), 1.97 (3H, dd, *J* = 5.6, 1.4 Hz; H-4″), 1.95 (3H, dd, *J* = 5.6, 1.4 Hz; H-4‴), 1.89 (3H, t, *J* = 1.4 Hz; H-5″, 1.85 (3H, t, *J* = 1.5 Hz; H-5‴), 1.17 (3H, d, *J* = 6.5 Hz; H-3); ^{13}C NMR, δ 167.2 (C-1‴), 166.7 (C-1″), 149.0 (C-5′), 143.6 (C-3′), 138.9 (C-3″), 138.1 (C-3‴), 135.4 (C-4′), 131.7 (C-1′), 127.8 (C-2″), 127.5 (C-2‴), 107.3 (C-6′), 101.7 (C-2′), 101.6 (C-7′), 77.1 (C-1), 71.2 (C-2), 56.6 (-OMe), 20.5 (C-5″), 20.5 (C-5‴), 16.8 (C-3), 15.8 (C-4″), 15.7 (C-4‴).

Compound 2 (2-epilaserine): colorless oil; ESI-MS, *m*/*z* 405 $[M + H]^+$; $[\alpha]_D^{25}$ –2.6° (*c* 0.1 in $CHCl_3$); ^{1}H NMR data, **Table 1**; ^{13}C

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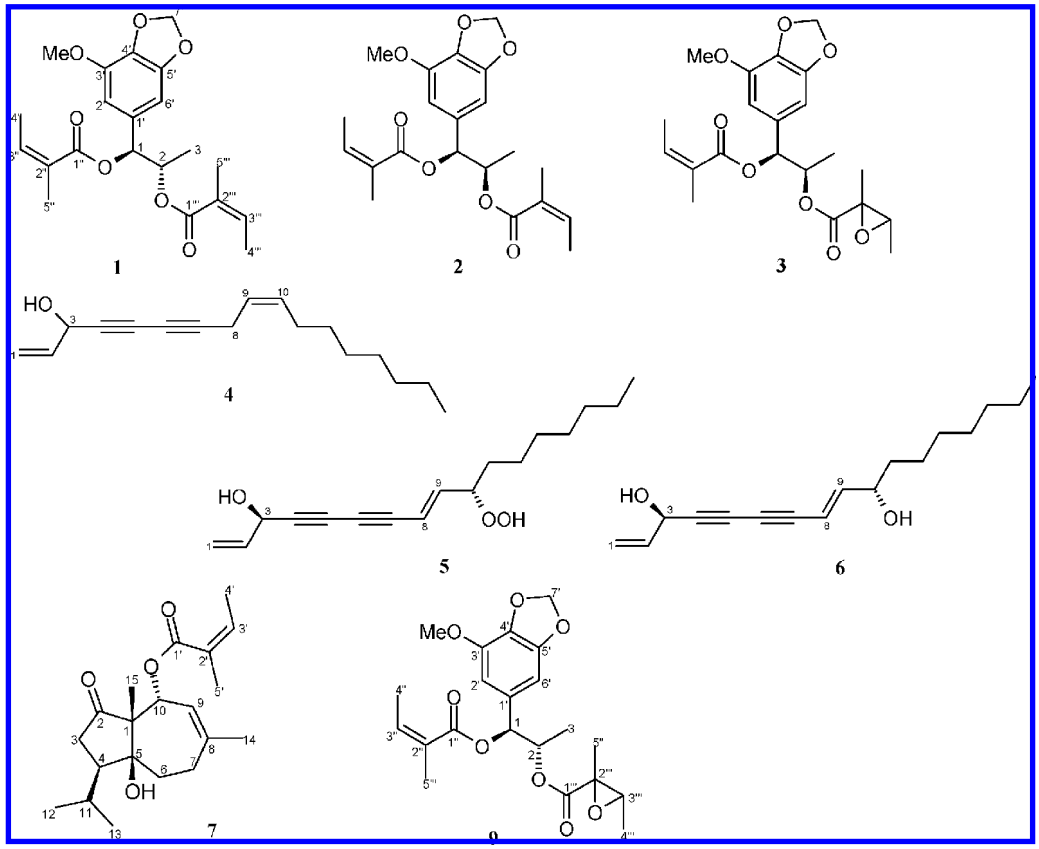

Figure 1. Structures of compounds **1**–**7** and **9**: (**1**) laserine; (**2**) 2-epilaserine; (**3**) epilaserine oxide; (**4**) panaxynol; (**5**) ginsenoyne K; (**6**) (8*E*)-1,8-heptadecadiene-4,6-diyne-3,10-diol; (**7**) vaginatin; (**9**) laserine oxide.

NMR, δ 167.1 (C-1‴), 166.5 (C-1″), 148.9 (C-5′), 143.4 (C-3′), 139.2 (C-3″), 138.2 (C-3‴), 133.0 (C-4′), 131.5 (C-1′), 127.7 (C-2″), 127.5 (C-2‴), 107.0 (C-6′), 101.5 (C-2′), 101.2 (C-7′), 75.9 (C-1), 71.6 (C-2), 56.6 (-OMe), 20.6 (C-5″), 20.5 (C-5‴), 15.8 (C-3), 15.7 (C-4″), 15.1 (C-4‴).

Compound 3 (epilaserine oxide): colorless oil; HRMS $[M + Na]^+$ calcd for $C_{21}H_{26}O_8Na$, *m/z* 429.1525, found, 429.1519; $[\alpha]_D^{25}$ –26° (*c* 0.075 in $CHCl_3$); NMR data, **Table 2**.

Compound 4 (panaxynol): colorless oil; ESI-MS, *m/z* 245 $[M + H]^+$; ^{1}H NMR, δ 5.94 (1H, ddd, J = 16.3, 10.2, 5.4 Hz; H-2), 5.52 (1H, dd, J = 9.1, 7.7 Hz; H-10), 5.46 (1H, d, J = 17.1 Hz; H-1a), 5.37 (1H, dd, J = 12.3, 7.1 Hz; H-9), 5.24 (1H, d, J = 10.1 Hz; H-1b), 4.91 (1H, d, J = 5.1 Hz; H-3), 3.03 (2H, d, J = 6.9 Hz; H-8), 2.02 (2H, dd, J = 14.4, 7.2 Hz; H-11), 1.36 (2H, t, J = 6.6 Hz), 0.88 (3H, t, J = 6.8 Hz; H-17); ^{13}C NMR, δ 136.2 (C-2), 133.1 (C-10), 121.9 (C-9), 117.0 (C-1), 80.3 (C-7), 74.3 (C-4), 71.3 (C-5), 64.0 (C-6), 63.5 (C-3), 31.8 (C-15), 29.2 (C-12), 29.2 (C-13), 29.1 (C-14), 27.2 (C-11), 22.6 (C-16), 17.9 (C-8), 14.1 (C-17).

Compound 5 (ginsenoyne K): colorless oil; ESI-MS, *m/z* 276 $[M + H]^+$; ^{1}H NMR, δ 8.38 (1H, s; −OOH), 6.25 (1H, dd, J = 16.0, 7.5 Hz; H-9), 5.96 (1H, ddd, J = 17.0, 10.3, 5.7 Hz; H-2), 5.78 (1H, d, J = 16.0 Hz; H-8), 5.48 (1H, d, J = 17.1 Hz; H-1a), 5.26 (1H, d, J = 10.2 Hz; H-1b), 4.98 (1H, d, J = 4.3 Hz; H-3), 4.36 (1H, dd, J = 13.9, 6.8 Hz; H-10), 0.88 (3H, t, J = 7.1 Hz; H-17); ^{13}C NMR, δ 146.2 (C-9), 136.0 (C-2), 117.2 (C-1), 111.7 (C-8), 85.7 (C-10), 81.0[a] (C-4), 74.1[a] (C-7), 70.6[a] (C-6), 70.9[a] (C-5), 63.6 (C-3), 32.2 (C-11), 31.7 (C-15), 29.4[b] (C-13), 29.1[b] (C-14), 25.1 (C-12), 22.6 (C-16), 14.0 (C-17). ([a, b]: assignments may be interchanged.)

Compound 6 [(8*E*)-1,8-heptadecadiene-4,6-diyne-3,10-diol): colorless oil; ESI-MS, *m/z* 260 $[M + H]^+$; ^{1}H NMR, δ 6.32 (1H, dd, J = 16.0, 5.3 Hz; H-9), 5.96 (1H, ddd, J = 17.0, 10.3, 5.7 Hz; H-2), 5.76 (1H, d, J = 15.9 Hz; H-8), 5.48 (1H, d, J = 17.1 Hz; H-1a), 5.26 (1H, d, J = 10.2 Hz; H-1b), 4.98 (1H, d, J = 4.3 Hz; H-3), 4.18 (1H, dt, J = 6.1, 6.1 Hz; H-10), 0.88 (3H, t, J = 7.1 Hz; H-17); ^{13}C NMR, δ 150.0 (C-9), 136.1 (C-2), 117.1 (C-1), 108.0 (C-8), 72.0 (C-10), 80.6[a] (C-4), 77.5[a] (C-7), 73.6[a] (C-6), 70.9[a] (C-5), 63.6 (C-3), 36.8 (C-11), 31.8 (C-15), 29.4[b] (C-13), 29.2[b] (C-14), 25.2 (C-12), 22.6 (C-16), 14.0 (C-17). ([a, b]: assignments may be interchanged.)

Compound 7 (vaginatin): colorless oil; ESI-MS, *m/z* 335 $[M + H]^+$; ^{1}H NMR, δ 6.02 (1H, ddd, J = 14.5, 7.3, 1.0 Hz; H-3′), 5.69 (1H, d, J = 7.7 Hz; H-9), 5.26 (1H, d, J = 7.7 Hz; H-10), 2.4 (2H, m, H-3,6), 2.27 (1H, ddd, J = 11, 10, 7 Hz; H-4), 2.25 (1H, m; H-7), 2.23 (1H, dd, J = 14, 7 Hz; H-3), 2.1 (2H, m; H-6, 7), 2.09 (1H, m; H-11), 1.97 (3H, dd, J = 7.2, 1.1 Hz; 5′-Me), 1.84 (3H, s; 4′-Me), 1.76 (3H, s; 14-Me), 1.06 (6H, t, J = 4.0 Hz; 13, 15-Me), 0.99 (3H, d, J = 6.7 Hz; 12-Me); ^{13}C NMR, δ 220.0 (C-2), 166.1 (C-1′), 146.1 (C-8), 139.3 (C-3′), 126.9 (C-2′), 119.7 (C-9), 82.3 (C-5), 75.7 (C-10), 60.1 (C-1), 50.8 (C-4), 38.5 (C-3), 37.1 (C-6), 29.1 (C-7), 26.4 (C-11), 26.1 (C-14), 24.6 (C-13), 21.1 (C-12), 20.7 (C-4′), 18.2 (C-15), 15.7 (C-5′).

Extraction Procedure for Comparative Studies. Carrots I (Jiangsu province, 250 g), II (Anhui province, 250 g), and III (Shanghai, 250 g) were squeezed with a Philip juice extractor and filtered. The three parts of residue and the juice of carrot III were extracted with 200 or 300 mL of petroleum ether (60–90 °C), respectively. Another carrot III (250 g) was divided into skin and center with a general parer. The center part was also squeezed with a Philip juice extractor. The residue of the center and the skin were extracted with 300 or 100 mL of petroleum ether (60–90 °C), respectively. After removal of the solvent in vacuo, all of the residues were dissolved in 5 mL of petroleum ether (60–90 °C) and filtered through a 0.45 μm Millipore nylon filter before HPLC.

Bioactivity Assays. A549 and HL-60 cells were obtained from the National Center for Drug Screening in Shanghai, China. Cytotoxicity assays of **1** and **2** were carried out by MTT colorimetric method.

Cells were seeded in 96-well microplates (1×10^4 cells/well) and routinely cultured in a humidified incubator (37 °C in 5% CO_2) for 24 h. **1** and **2** were added in serial concentrations and reincubated for 48 h. Then 10 μL of tetrazolium dye (MTT) solution (5 mg/mL in PBS) was added to each well, and the solution was incubated for an

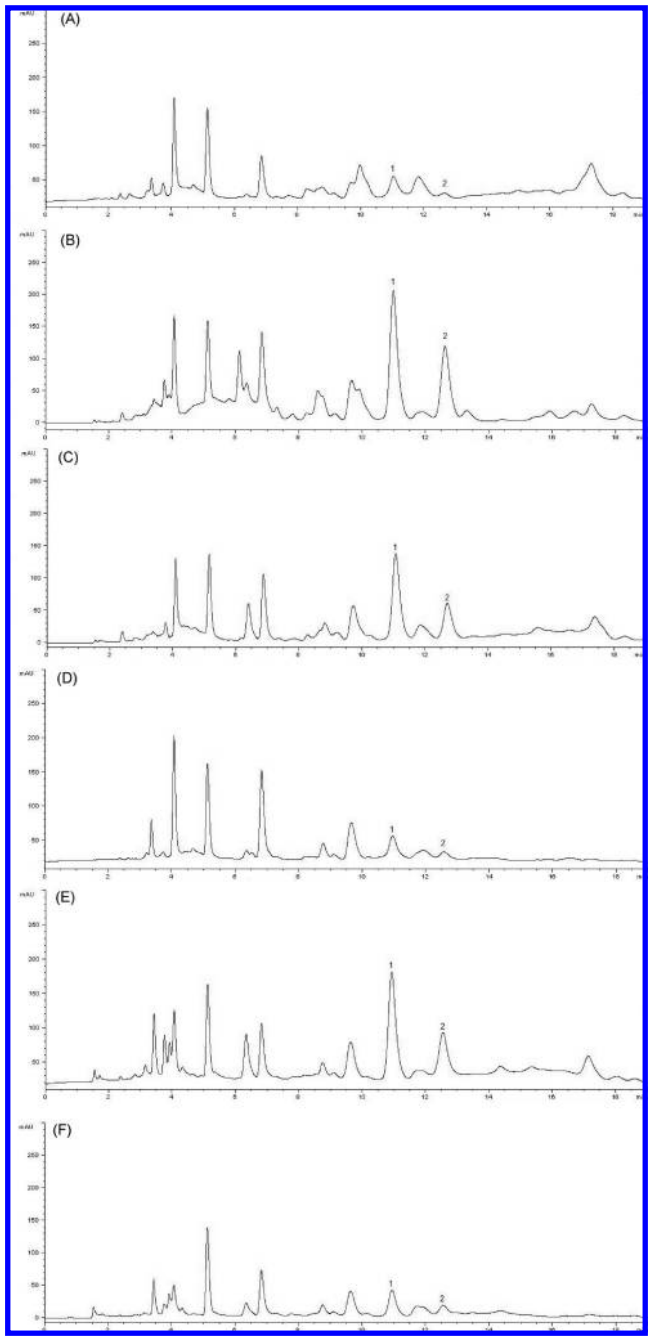

Figure 2. HPLC chromatograms at 220 nm of extracts of different carrots: (**A**) carrot I; (**B**) carrot II; (**C**) carrot III; (**D**) carrot III skin; (**E**) carrot III center; (**F**) carrot III juice. Peaks: 1, laserine; 2, 2-epilaserine.

additional 4 h. Then the medium was discarded, and 100 μL of DMSO was added to dissolve the formazan crystals. The plate was read on a microplate reader at 490 nm. MTT solution with DMSO (without cells and medium) acted as a blank control in microplate reading.

RESULTS AND DISCUSSION

Isolation and Structure Elucidation of Compounds. The Et_2O extract of carrot was subjected to silica gel column chromatography and HPLC to give **1**−**7**.

Compounds **1** and **2** were identified as laserine and 2-epilaserine (C-2 epimers) by comparison of the spectroscopic data with those of the known compounds (*8*). The extraction and isolation of **1** and **2** were taken under ambient conditions. Under such conditions, they are stable, and no epoxidation of either was observed.

Table 1. ^{1}H NMR Data of Compounds **8**, **2**,**3**, and Laserine Oxide (**9**)

C/H	8[a]	2	3	9 (*13*)
1	5.80 (d, 4.8)	5.92 (d, 4.4)	5.86 (d, 4.4)	5.76 (d, 7.0)
2	5.20 (dq, 6.5, 4.8)	5.29 (dd, 6.4, 4.4)	5.30 (dq, 6.5, 4.8)	5.35 (d, 7.0)
3	1.20 (d, 6.5)	1.27 (d, 6.5)	1.27 (d, 6.5)	1.20 (d, 6.5)
2′	6.51 (s)	6.58 (s)	6.58 (s)	6.60 (s)
6′	6.52 (s)	6.56 (s)	6.57 (s)	6.60 (s)
7′	5.95 (s)	5.96 (s)	5.96 (s)	5.97 (s)
3″	2.94 (q)	6.13 (qq, 7.3, 1.4)	6.14 (qq, 7.0, 1.4)	6.15 (qq, 7.0, 1.0)
4″	1.10 (d, 5.5)	2.00 (dd, 7.3, 1.5)	2.00 (dd, 7.0, 1.5)	1.99 (dq, 7.0, 1.0)
5″	1.40 (s)	1.96 (t, 1.4)	1.95 (t, 1.4)	1.96 (d, 1.0)
3‴	6.06 (qq, 7.2, 1.4)	6.04 (qq, 7.3, 1.4)	3.02 (dq, 5.4)	3.00 (q, 5.5)
4‴	1.93 (dq, 1.4, 1.4)	1.93 (dd, 7.3, 1.5)	1.24 (d, 5.4)	1.22 (d, 5.5)
5‴	1.80 (dd, 7.2, 1.4)	1.84 (t, 1.4)	1.47 (br s)	1.53 (s)

[a] ^{1}H NMR data were reported by Saouf et al. (*12*), but their assignments were corrected.

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Epilaserine Oxide (**3**) in $CDCl_3$

C/H	δ_H (mult, *J* in Hz)	δ_C	COSY	HMBC
1	5.86 (d, 4.4)	75.8	H-2	C-1″, C-1′, C-6′, C-2′, C-2, C-3
2	5.30 (dd, 6.4, 4.4)	73.0	H-1, H-3	C-1‴, C-1, C-3
3	1.27 (d, 6.5)	15.6	H-2	C-2, C-3
1′		131.1		
2′	6.58 (br s)	101.5		C-3′, C-4′, C-5′, C-6′, C-1
3′		143.5		
4′		135.1		
5′		149.0		
6′	6.57 (br s)	107.4		C-3′, C-4′, C-5′, C-6′, C-1
7′	5.96 (s)	101.6		C-3′, C-4′
1″		166.7		
2″		127.3		
3″	6.14 (qq, 7.0, 1.3)	139.4	H-4″, H-5″	
4″	2.00 (dd, 7.0, 1.5)	15.9	H-3″, H-5″	C-2″, C-3″
5″	1.95 (t, 1.4)	20.6	H-3″, H-4″	C-1″, C-2″, C-3″
1‴		168.2		
2‴		59.8		
3‴	3.02 (dq, 5.4)	59.8	H-4‴	C-5‴
4‴	1.24 (d, 5.4)	13.5	H-3‴	C-2‴, C-3‴
5‴	1.47 (s)	19.1		C-1‴, C-2‴, C-3‴
−OMe	3.90 (s)	56.7		C-5′

Compound **3** was obtained as a colorless oil. The ^{1}H NMR data (**Table 1**) of **3** revealed it should be a derivative of **1** or **2**. The chemical shift of H-1 (δ 5.92) and the coupling constant between H-1 and H-2 ($J_{1,2}$ = 4.4 Hz) of **3** suggested an *erythro* configuration of the diol system as in the case of **2**. Therefore, it should be derivative of 2-epilaserine. The NMR data of C-1′−C-7′ and C-1″−C-5″ in **3** and **2** demonstrated the identical substituent at C-1 of the two compounds. The NMR data of C-1‴−C-5‴ of **3** and **2** showed the double bond between C-2‴ and C-3‴ in **2** was oxidized to an epoxy group in **3**, which was confirmed by 2D-NMR data. Therefore, **3** was deduced as epilaserine oxide. It was a new compound.

Compounds **4**−**7** were identified by comparison of their spectroscopic data with those of the known compounds (*9*–*11*).

Correct Structure of 8. Recently, **8**, along with other new phenylpropanoids from *Thapsia transtagana*, was reported (*12*) (**Figure 3**). After comparing the NMR data of **8** with those of **2** and **3**, we found the reported structure of **8** was misassigned (**Table 1**).

The chemical shifts of H-3″, H-4″, H-5″ and H-3‴, H-4‴, H-5‴ of two angeloyls in **2** were found to be different. On the basis of ^{1}H NMR data of **8** the reported chemical shifts of angeloyl were the same as H-3‴, H-4‴, H-5‴ but different from H-3″, H-4″, H-5″ of **2**, which indicated the oxidized double bond should be located at position C-2″ instead of C-2‴. If

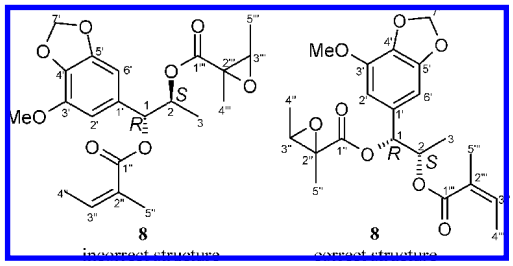

Figure 3. Correct structure of compound **8**.

C-2‴ and C-3‴ were oxidized, the chemical shifts of H-3″, H-4″, and H-5″ should not be affected. This conclusion was also confirmed by comparing ^{1}H NMR data of **3** with the reported compound laserine oxide (**9**) (*13*); therefore, the correct structure of **8** should be the molecule shown in **Figure 3**.

Bioactivity of 1 and 2. Although phenylpropanoids are common in the Umbelliferae family, none of these compounds from carrot was previously reported. Laserine was a phenylpropanoid with angeloyl found mainly in the genus *Ferula* and 2-epilaserine was merely reported from *Seseli vayredanum*. As far as we know, there was no activity report on laserine (**1**) and 2-epilaserine (**2**).

2-Epilaserine (**2**) displayed significant cytotoxicity against HL-60 cells, whereas laserine (**1**) displayed only weak cytotoxic activity. The IC_{50} of **2** was 0.52 μM (95% confidence interval $= 8.52 \times 10^{-2}-3.92$). Both **1** and **2** showed no cytotoxic activity against A549 and PC12 cell lines.

Distribution of Laserine and 2-Epilaserine in Carrots from Different Sections. Because 2-epilaserine (**2**) displayed significant cytotoxicity against HL-60 cells, we wondered if this compound was an inherent secondary metabolite of carrot. Therefore, RP-HPLC was used to analyze laserine and 2-epilaserine in different carrot cultivars and root sections.

HPLC results showed all of the carrots gathered from three locations contained laserine and 2-epilaserine (**Figure 2**). Both laserine and 2-epilaserine were detected in each section, that is, juice, skin, and center, which implied that the cytotoxic 2-epilaseine was taken with other nutrient components whether by the drinking of carrot juice or the ingestion of carrot even without skin.

As we reported here, a new phenylpropanoid, epilaserine oxide (**3**), was obtained from carrot, and the identification of its structure led to the modification of the structure of another phenylpropanoid (**8**) previously reported. On the other hand, phenylpropanoids with angeloyl, especially laserine and 2-epilaserine, were inherent secondary metabolites of carrot besides polyacetylene, and 2-epilaserine was one of the cytotoxic components of carrot. This result indicated that the bioactivity of phenylpropanoids with angeloyl needs to be further investigated, which may be used to explain some of the health-promoting properties of carrot and other Umbelliferae plants. Furthermore, the toxicity of phenylpropanoids with angeloyl should be investigated to confirm the safety of carrot.

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