

# Physical Stability of Shikonin Derivatives from the Roots of *Lithospermum erythrorhizon* Cultivated in Korea

Man-Ho Cho,<sup>†</sup> Young-Sook Paik,<sup>‡</sup> and Tae-Ryong Hahn<sup>\*†</sup>

Departments of Genetic Engineering and Chemistry, Kyung Hee University, Suwon 449-701, Korea

Five red shikonin pigments, deoxyshikonin, shikonin, acetylshikonin, isobutylshikonin, and  $\beta$ -hydroxyisovalerylshikonin, were isolated from the roots of *Lithospermum erythrorhizon* cultivated in Korea. The purified pigments were red, purple, and blue at acidic, neutral, and alkaline pH values, respectively. Physical stability of the purified pigments against heat and light in an aqueous solution was examined for possible value-added food colorants. The thermal degradation reactions were carried out at pH 3.0 (50 mM glycine buffer) in 50% EtOH/H<sub>2</sub>O. Deoxyshikonin ( $t_{1/2}$  = 14.6 h, 60 °C) and isobutylshikonin ( $t_{1/2}$  = 19.3 h, 60 °C) are relatively less stable than other shikonin derivatives ( $t_{1/2}$  = 40–50 h, 60 °C). Activation energies of thermal degradation of the isolated pigments were calculated. The activation energy of deoxyshikonin was the highest (12.5 kcal mol<sup>-1</sup>) and that of  $\beta$ -hydroxyisovalerylshikonin was the lowest (1.71 kcal mol<sup>-1</sup>) value. Light stabilities of the pigments were similar to each other in that the half-life values of photodegradation for 20000 lx light intensity were 4.2–5.1 h.

**Keywords:** *Lithospermum erythrorhizon*; shikonin derivatives; naphthoquinones; physical stability

## INTRODUCTION

The root of *Lithospermum erythrorhizon* has been used as a drug for healing burns, inflammation, and wounds (Tanaka et al., 1986) and as a dye for staining fabrics and food colorants. Red pigments of *L. erythrorhizon* accumulate in the cork layers of the roots of the plant (Tabata et al., 1974). The color components of *L. erythrorhizon* are composed of naphthoquinone compounds: shikonin and its derivatives (Morimoto et al., 1965; Morimoto and Hirata, 1966; Kyogoku et al., 1973; Mizukami et al., 1978). The contents and composition of shikonin pigments are different with species and cultivated areas (Kyogoku et al., 1973; Tsukada et al., 1983).

The stability of shikonin pigments during processing and storage is crucial to their use as drugs, cosmetics, and food colorants. Factors influencing color stability are heat, light, oxygen, and pH (Attoe and von Elbe, 1981). The colors of the shikonin pigments sharply change at different pH values (Chung and Lee, 1994). Isobutylshikonin and acetylshikonin are reported as stable against heat in an acidic condition (Chung and Lee, 1995). However detailed kinetic data for thermal and light stability of shikonin derivatives are yet to be determined.

In this research, we isolated five shikonin pigments from *L. erythrorhizon* cultivated in Korea with silica gel column chromatography and preparative TLC. The isolated pigments were identified by NMR and CIMS spectrometry. Experiments to determine the heat stability of the isolated pigments were performed at pH 3.0 in 50% EtOH/H<sub>2</sub>O. The stability of the pigments

against light was also determined under different light intensities at room temperature.

## EXPERIMENTAL PROCEDURES

**Materials.** The roots of *L. erythrorhizon* were purchased from a local market and stored at room temperature. Silica gel 60 for column chromatography and TLC plates were obtained from Merck. Reagents for buffer preparation including sodium phosphate, glycine, 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES), and sodium citrate were purchased from Sigma Chemical Co. Other chemicals including hexane, ethyl acetate (EtOAc), and ethanol (EtOH) were obtained from Hayman Ltd. and Tedia Co., Inc.

**Spectral Analysis.** The UV-vis spectra of the isolated pigments were obtained with a Varian DMS 300 spectrophotometer. The isolated pigments dissolved in EtOH were scanned in the range of 200–700 nm. The <sup>1</sup>H NMR, <sup>1</sup>H–<sup>1</sup>H COSY, <sup>13</sup>C NMR, and <sup>1</sup>H–<sup>13</sup>C COSY spectra of the isolated pigments were measured in CDCl<sub>3</sub> on a 400 MHz FT-NMR (JEOL) at 400 and 100 MHz, respectively. CIMS spectra were obtained with a JEOL JMS-AS505 WA mass spectrometer.

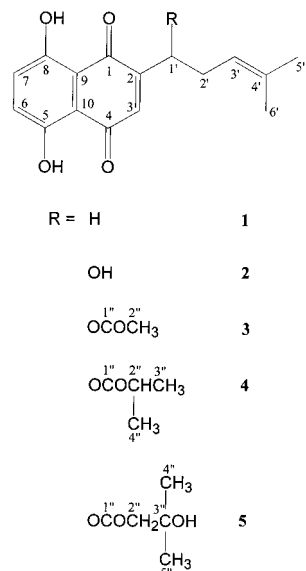
**Extraction and Isolation.** Roots of *L. erythrorhizon* (200 g) were extracted with 2 L of hexane at room temperature for 12 h with stirring. The extracts were filtered through Whatman No. 2 paper and concentrated to a small volume (20 mL). The concentrates were applied to a silica gel column. The pigments were eluted gradiently with a hexane/EtOAc mixture from 20:1 to 1:1. Five fractions were obtained from the column. Two fractions were further subjected to preparative TLC developed with 20:1 and 5:1 mixtures of hexane/EtOAc, yielding pigments **1** (3 mg) and **2** (16 mg), respectively. Three other fractions were concentrated and applied to a silica gel column for further purification and eluted with 20:1, 5:1, and 3:1 mixtures of hexane/EtOAc, yielding pigments **4** (100 mg), **3** (240 mg), and **5** (120 mg), respectively. The isolated pigments were identified with UV, NMR, and MS spectrometry (Figure 1).

**Pigment 1 (deoxyshikonin):** UV  $\lambda_{\max}^{\text{EtOH}}$  277, 485, 514, 552 nm; CIMS, 70 eV,  $m/z$  273 [ $M + 1$ ]<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, TMS)  $\delta$  1.60 (3H, s, H-6'), 1.70 (3H, s, H-5'), 2.31 (2H, m, H-2'), 2.64 (2H, t,  $J$  = 7.6 Hz, H-1'), 5.14 (1H, t,  $J$  = 7.2 Hz,

\* Author to whom correspondence should be addressed (telephone 82-331-201-2434; fax 82-331-202-2687; e-mail trhahn@nms.kyunghee.ac.kr).

<sup>†</sup> Department of Genetic Engineering.

<sup>‡</sup> Department of Chemistry.



**Figure 1.** Chemical structures of the isolated pigments from the roots of *L. erythrorhizon* cultivated in Korea. The isolated pigments were identified with <sup>1</sup>H and <sup>13</sup>C NMR, <sup>1</sup>H-<sup>1</sup>H and <sup>1</sup>H-<sup>13</sup>C COSY, and CIMS spectra as deoxyshikonin (**1**), shikonin (**2**), acetylshikonin (**3**), isobutylshikonin (**4**), and β-hydroxyisovalerylshikonin (**5**).

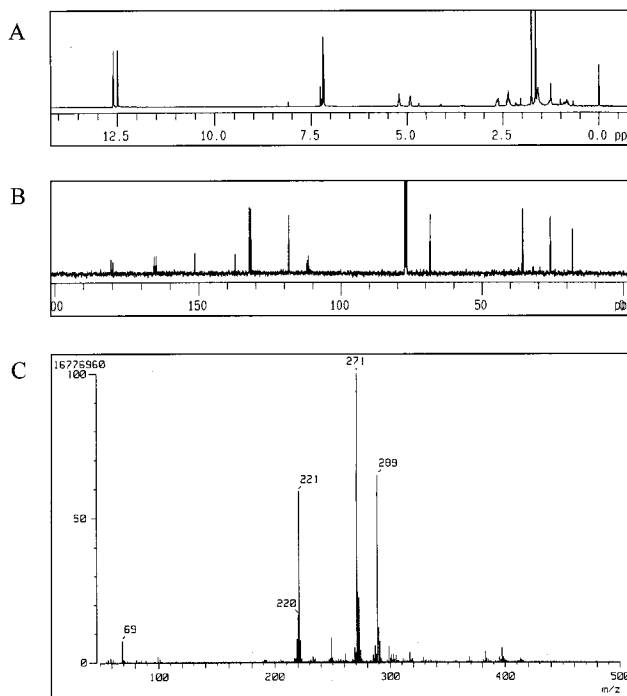
H-3'), 6.84 (1H, s, H-3), 7.20 (2H, s, H-6 and H-7), 12.46 and 12.62 (2H, s, OH); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, TMS) δ 17.8 (C-6'), 25.6 (C-5'), 26.5 (C-2'), 29.7 (C-1'), 111.7 and 111.9 (C-9 and C-10), 122.3 (C-3'), 130.9 and 131.1 (C-6 and C-7), 133.6 (C-4'), 134.5 (C-3), 151.5 (C-2), 162.3 and 162.9 (C-5 and C-8), 183.0 (C-1 and C-4).

**Pigment 2 (shikonin):** UV λ<sub>max</sub><sup>EtOH</sup> 278, 486, 516, 554 nm; CIMS, 70 eV, *m/z* 289 [M + 1]<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, TMS) δ 1.66 (3H, s, H-6'), 1.76 (3H, s, H-5'), 2.36 and 2.65 (2H, m, H-2'), 4.92 (1H, dd, *J* = 4.1 and 7.6 Hz, H-1'), 5.21 (1H, dd, *J* = 7.3 Hz, H-3'), 7.17 (1H, s, H-3), 7.20 (2H, d, *J* = 2.4 Hz, H-6 and H-7), 12.49 and 12.60 (2H, s, OH); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, TMS) δ 18.1 (C-6'), 25.9 (C-5'), 35.7 (C-2'), 68.4 (C-1'), 111.6 and 112.0 (C-9 and C-10), 118.5 (C-3'), 131.9 (C-3), 132.3 and 132.4 (C-6 and C-7), 137.4 (C-4'), 151.4 (C-2), 164.9 and 165.6 (C-5 and C-8), 179.8 and 180.6 (C-1 and C-4).

**Pigment 3 (acetylshikonin):** UV λ<sub>max</sub><sup>EtOH</sup> 275, 489, 520, 560 nm; CIMS, 70 eV, *m/z* 331 [M + 1]<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, TMS) δ 1.58 (3H, s, H-6'), 1.70 (3H, s, H-5'), 2.14 (3H, s, H-2''), 2.47 and 2.62 (2H, m, H-2'), 5.12 (1H, dd, *J* = 7.3 Hz, H-3'), 6.02 (1H, dd, *J* = 4.6 and 7.1 Hz, H-1'), 6.99 (1H, s, H-3), 7.18 (2H, s, H-6 and H-7), 12.42 and 12.58 (2H, s, OH); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, TMS) δ 17.9 (C-6'), 25.8 (C-5'), 32.8 (C-2'), 69.5 (C-1'), 111.5 and 111.8 (C-9 and C-10), 117.7 (C-3'), 131.4 (C-3), 132.5 and 132.7 (C-6 and C-7), 136.1 (C-4'), 148.2 (C-2), 167.0 and 167.5 (C-5 and C-8), 169.7 (C-1'), 176.7 and 178.2 (C-1 and C-4).

**Pigment 4 (isobutylshikonin):** UV λ<sub>max</sub><sup>EtOH</sup> 275, 488, 520, 561 nm; CIMS, 70 eV, *m/z* 359 [M + 1]<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, TMS) δ 1.22 (6H, d, *J* = 7.1 Hz, H-3'' and H-4''), 1.59 (3H, s, H-6'), 1.69 (3H, s, H-5'), 2.47 and 2.64 (2H, m, H-2'), 2.65 (1H, q, *J* = 7.1 Hz, H-2''), 5.13 (1H, dd, *J* = 7.3 Hz, H-3'), 6.02 (1H, dd, *J* = 4.6 and 7.2 Hz, H-1'), 6.97 (1H, s, H-3), 7.16 (2H, s, H-6 and H-7), 12.40 and 12.56 (2H, s, OH); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, TMS) δ 17.9 (C-6'), 18.8 and 18.9 (C-3'' and C-4''), 25.7 (C-5'), 32.9 (C-2'), 34.0 (C-2''), 69.0 (C-1'), 111.5 and 111.8 (C-9 and C-10), 117.8 (C-3'), 131.3 (C-3), 132.5 and 132.7 (C-6 and C-7), 135.9 (C-4'), 148.5 (C-2), 166.7 and 167.2 (C-5 and C-8), 175.7 (C-1'), 176.8 and 178.3 (C-1 and C-4).

**Pigment 5 (β-hydroxyisovalerylshikonin):** UV λ<sub>max</sub><sup>EtOH</sup> 276, 489, 520, 560 nm; CIMS, 70 eV, *m/z* 388 [M + 1]<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, TMS) δ 1.30 and 1.31 (6H, s, H-4'' and H-5''), 1.59 (3H, s, H-6'), 1.69 (3H, s, H-5'), 2.51 and 2.62 (2H, m, H-2'), 2.59 (2H, s, H-2''), 5.12 (1H, dd, *J* = 6.7 Hz, H-3'), 6.10



**Figure 2.** <sup>1</sup>H (A) and <sup>13</sup>C NMR (B) and CIMS (C) spectra of pigment **2** (shikonin). The <sup>1</sup>H and <sup>13</sup>C NMR spectra of shikonin were measured in CDCl<sub>3</sub> at 400 and 100 MHz, respectively.

(1H, dd, *J* = 4.6 and 7.2 Hz, H-1'), 7.03 (1H, s, H-3), 7.27 (2H, s, H-6 and H-7), 12.40 and 12.59 (2H, s, OH); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, TMS) δ 17.9 (C-6'), 25.7 (C-5'), 29.1 and 29.2 (C-4'' and C-5''), 32.9 (C-2'), 46.5 (C-2''), 69.1 (C-3'), 69.8 (C-1'), 111.6 and 111.8 (C-9 and C-10), 117.6 (C-3'), 131.3 (C-3), 133.1 and 133.3 (C-6 and C-7), 136.4 (C-4'), 147.5 (C-2), 168.1 and 168.7 (C-5 and C-8), 171.6 (C-1'), 175.4 and 176.9 (C-1 and C-4).

**UV-Vis Spectra at Different pH Values.** UV-vis spectral changes of the pigments were measured at different pH values using various buffer systems. Buffer systems used were 50 mM phosphate (pH 2.0, 7.0, and 12.0), 50 mM citrate (pH 5.0), and 50 mM CHES (pH 9.0) in 50% EtOH/H<sub>2</sub>O. UV-vis spectra of the pigments were measured at the range of 200–700 nm.

**Sample Preparation and Reaction Condition.** Each pigment was dissolved in 50% EtOH/H<sub>2</sub>O at pH 3.0 (50 mM glycine buffer). Pigment concentrations were adjusted to give an initial absorbance between 0.7 and 0.8 at λ<sub>vis,max</sub>. Degradation reactions were carried out in 1.5 mL quartz spectrophotometer cuvettes. The prepared samples were degraded at temperatures of 40–70 °C. Duplicate tests of thermal degradation reaction were performed. Photodegradation of the isolated pigments was carried out in a phytotron (Vision Co.) at 25 °C with different light intensities of 5000–20000 lx. Light intensities were adjusted with a lux meter, and sample cuvettes were placed on it. Thermal degradation and photodegradation of the isolated pigments were monitored spectrophotometrically at λ<sub>vis,max</sub>. The reaction was monitored for more than one half-life.

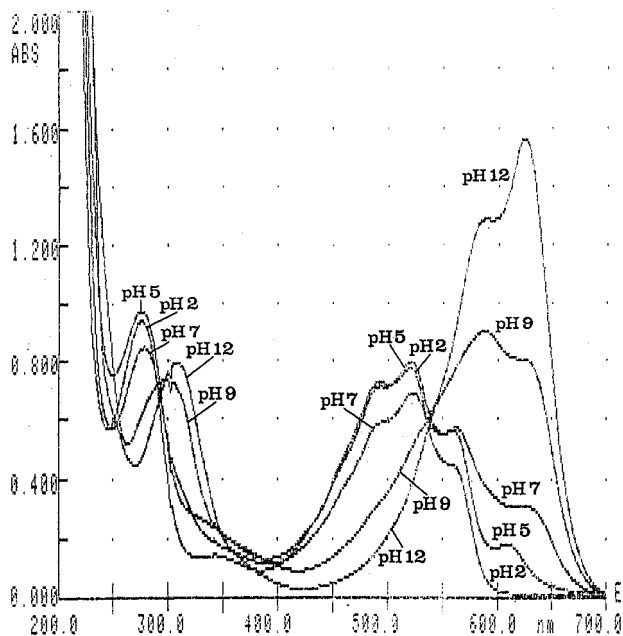
## RESULTS AND DISCUSSION

Five red pigments were isolated from *L. erythrorhizon* cultivated in Korea with silica gel column chromatography and preparative TLC. UV-vis measurements of the isolated pigments showed typical UV-vis spectra of shikonin pigments, indicating that the isolated pigments were shikonin and its derivatives. The isolated pigments were identified with <sup>1</sup>H and <sup>13</sup>C NMR, <sup>1</sup>H-<sup>1</sup>H and <sup>1</sup>H-<sup>13</sup>C COSY, and CIMS as deoxyshikonin, shikonin, acetylshikonin isobutylshikonin, and β-hy-

**Table 1. Thermal Degradation Rate Constants (*k*) and Half-Life Values (*t*<sub>1/2</sub>) for the Isolated Pigments in 50% EtOH/H<sub>2</sub>O at pH 3.0 (50 mM Glycine Buffer) at Different Temperatures<sup>a</sup>**

temp (°C)	<i>k</i> (s <sup>-1</sup> × 10 <sup>6</sup> )					<i>t</i> <sub>1/2</sub> (h)				
	1	2	3	4	5	1	2	3	4	5
40	4.17 ± 1.85	2.49 ± 0.16	2.87 ± 0.49	6.36 ± 0.25	2.94 ± 0.02	46.2 ± 21.2	77.4 ± 5.4	67.0 ± 9.6	30.3 ± 1.2	65.4 ± 0.5
50	8.22 ± 1.82	2.93 ± 0.69	3.55 ± 0.33	7.85 ± 1.70	3.24 ± 0.40	23.4 ± 6.7	65.6 ± 12.4	54.2 ± 4.6	24.5 ± 4.3	59.4 ± 8.4
60	13.2 ± 2.5	3.55 ± 0.07	4.79 ± 0.03	9.98 ± 0.59	3.51 ± 0.35	14.6 ± 2.4	54.2 ± 1.2	40.2 ± 0.3	19.3 ± 1.2	54.8 ± 6.2
70	24.9 ± 3.3	4.41 ± 0.20	6.15 ± 0.59	12.8 ± 3.03	3.74 ± 0.12	7.73 ± 1.2	43.7 ± 2.1	31.3 ± 3.3	15.1 ± 4.6	51.4 ± 1.8

<sup>a</sup> 1, deoxyshikonin; 2, shikonin; 3, acetylshikonin; 4, isobutylshikonin; 5, β-hydroxyisovalerylshikonin.



**Figure 3.** UV-vis spectra of pigment 3 (acetylshikonin) at different pH values. The color of acetylshikonin was red, purple, and blue at acidic, neutral, and alkaline pH values in 50% EtOH/H<sub>2</sub>O, respectively. Buffer systems used were 50 mM phosphate (pH 2.0, 7.0, and 12.0), 50 mM citrate (pH 5.0), and 50 mM CHES (pH 9.0).

droxyisovalerylshikonin. Figure 2 shows the <sup>1</sup>H and <sup>13</sup>C NMR and CIMS spectra of pigment 2. Two proton peaks of phenolic OH appeared at δ 12.49 and 12.60 in the <sup>1</sup>H NMR spectrum (Figure 2A) of pigment 2. Two aromatic proton peaks appeared at δ 7.20 (2H, H-6 and H-7) and δ 7.17 (1H, H-3). Proton peaks at δ 5.21 (1H) and δ 4.92 (1H) were assigned as H-3' and H-1', respectively. Two protons of H-2' appeared at δ 2.65 (1H) and δ 2.36 (1H). Two proton peaks at δ 1.76 (3H) and δ 1.66 (3H) were assigned as H-5' and H-6', respectively. The <sup>13</sup>C NMR spectrum (Figure 2B) of pigment 2 showed 16 carbon peaks. The molecular ion peak [M + 1]<sup>+</sup> at *m/z* 289 in the CIMS spectrum (Figure 2C) of pigment 2 agreed with the molecular formula of shikonin (C<sub>16</sub>H<sub>16</sub>O<sub>5</sub>). Therefore, pigment 2 was identified as shikonin. So far, nine shikonin derivatives have been isolated and identified (Morimoto et al., 1965; Morimoto and Hirata, 1966; Kyogoku et al., 1973; Mizukami et al., 1978), and many acyl derivatives of shikonin have been synthesized (Ahn et al., 1995). <sup>1</sup>H NMR data of pigments 4 and 5 agreed with those of isobutylshikonin isolated by Morimoto et al. (1965) and β-hydroxyisovalerylshikonin isolated by Morimoto and Hirata (1966), respectively. <sup>1</sup>H and <sup>13</sup>C NMR data of pigments 3 and 4 were essentially identical with those of acyl derivatives of shikonin synthesized by Ahn et al. (1995). Because no literature data were available, pigment 1 was identified by analysis of the NMR and CIMS spectra. Two distinct singlet peaks at δ 12.46 and δ 12.62 in <sup>1</sup>H NMR spectra of the isolated

pigments were assigned as phenolic protons. Two aromatic proton peaks at δ 7.20 and δ 6.84 were two protons of H-6,7 and one proton of H-3, respectively. Triplet peaks at δ 5.14 were assigned as H-3' by analyzing the <sup>1</sup>H-<sup>13</sup>C COSY spectra. Two singlet peaks at δ 1.70 and δ 1.60 were assigned as H-5' and H-6', respectively, by analyzing the <sup>1</sup>H-<sup>13</sup>C COSY spectra. By analysis of <sup>1</sup>H-<sup>1</sup>H COSY spectra, the peaks at δ 2.31 were assigned as H-2'. The proton signal of H-1' (2H) at δ 2.64 was assigned by analysis of the <sup>1</sup>H-<sup>1</sup>H COSY spectrum. The <sup>13</sup>C signal of C-1' was observed at δ 29.7, whereas the corresponding peaks of pigments 2-5 were at δ 68.4-69.8, indicating that the oxygen atom does not attach C-1' of pigment 1. The molecular ion peak [M + 1]<sup>+</sup> at *m/z* 273 in the CIMS spectrum of pigment 1 agreed with the molecular formula of C<sub>16</sub>H<sub>16</sub>O<sub>4</sub>. Therefore, pigment 1 was identified as deoxyshikonin. In this research, we isolated five shikonin pigments and presented detailed spectral data for <sup>1</sup>H and <sup>13</sup>C NMR and UV-vis spectral data in EtOH.

The color of shikonin and its derivatives sharply changes at different pH values (Chung and Lee, 1994). The isolated pigments showed red, purple, and blue colors at acidic, neutral, and alkaline pH values in 50% EtOH/H<sub>2</sub>O, respectively. Figure 3 shows UV-vis spectra of acetylshikonin at different pH values.

The stability of pigments is affected by many factors such as heat, light, pH, and oxygen (Attoe and von Elbe, 1981). Thermal degradation reactions of the isolated pigments were carried out at pH 3.0 (50 mM glycine buffer) in 50% EtOH/H<sub>2</sub>O at different temperature ranges of 40-70 °C. The degree of degradation was determined by measuring absorbance changes at λ<sub>max</sub>. When the degree of degradation was plotted on a semilogarithmic scale, the plots showed a straight line, indicating that the reaction follows first-order kinetics. Rate constants and half-life values of thermal degradation reactions of the isolated pigments are summarized in Table 1. Rate constants and half-life values of the isolated pigments suggested that heat stabilities of the pigments were different with substituted groups. Isobutylshikonin and deoxyshikonin were the least stable at low (*t*<sub>1/2</sub> = 30 h, 40 °C) and high temperatures (*t*<sub>1/2</sub> = 7.7 h, 70 °C), respectively. Other shikonins were stable at experimental temperature ranges (*t*<sub>1/2</sub> = 54-65 h, 50 °C). The shikonin pigments were relatively stable compared with other natural pigments such as anthocyanins and betacyanins. The half-life values of anthocyanin pigments (cyanidin 3-arabinoside, cyanidin 3-galactoside, peonidin 3-arabinoside, and peonidin 3-galactoside) are 40-44 h at 55 °C in an acidic condition (Attoe and von Elbe, 1981). The half-life value of cyanidin 3-glucoside is 50 h at 70 °C in acidic condition (Cho et al., 1996). The half-life value of betacyanin from prickly pear is 3 h at 50 °C (Merin et al., 1987). Activation energies of the thermal degradation reactions of the isolated pigments are summarized in Table 2. Deoxy-

**Table 2. Activation Energies ( $E_a$ ) of the Thermal Degradation Reactions of the Isolated Pigments in 50 % EtOH/H<sub>2</sub>O at pH 3.0 (50 mM Glycine Buffer)**

	activation energy (kcal mol <sup>-1</sup> )
deoxyshikonin	12.5
shikonin	4.06
acetylshikonin	5.51
isobutylshikonin	4.91
$\beta$ -hydroxyisovalerylshikonin	1.71

**Table 3. Photodegradation Rate Constants ( $k$ ) and Half-Life Values ( $t_{1/2}$ ) for the Isolated Pigments in 50% EtOH/H<sub>2</sub>O at pH 3.0 (50 mM Glycine Buffer) under Different Light Intensities at 25 °C<sup>a</sup>**

light intensity (lx)	$k$ (s <sup>-1</sup> × 10 <sup>5</sup> )					$t_{1/2}$ (h)				
	1	2	3	4	5	1	2	3	4	5
5000	2.01	1.46	1.60	1.36	1.34	9.59	13.2	12.0	14.1	14.3
10000	2.88	2.53	2.78	2.37	2.44	6.67	7.60	6.92	8.11	7.90
15000	3.33	3.15	3.70	3.18	3.22	5.78	6.12	5.21	6.06	5.99
20000	3.78	3.83	4.58	4.19	4.13	5.08	5.03	4.21	4.59	4.65

<sup>a</sup> 1, deoxyshikonin; 2, shikonin; 3, acetylshikonin; 4, isobutylshikonin; 5,  $\beta$ -hydroxyisovalerylshikonin.

shikonin and  $\beta$ -hydroxyisovalerylshikonin showed the highest (12.5 kcal mol<sup>-1</sup>) and lowest (1.71 kcal mol<sup>-1</sup>) activation energy values, respectively. This result indicated that deoxyshikonin is most affected and  $\beta$ -hydroxyisovalerylshikonin is least affected by the increase of temperatures on thermal degradation of the pigments as shown in Table 1. Other shikonin derivatives showed similar activation energies of 4.1–5.5 kcal mol<sup>-1</sup>.

The photodegradation reactions of the isolated pigments were carried out at different light intensities of 5000–20000 lx at pH 3.0. Photodegradation reactions of the isolated pigments also followed first-order kinetics. The rate constants and half-life values of the photodegradation reactions of the isolated pigments were almost identical regardless of substituted groups (Table 3), suggesting that the substituted groups do not affect the photolysis of the naphthoquinone backbone. This result is in contrast to thermal degradation of the pigments, in which the thermal stability of shikonin pigments varied with the substituted groups (Table 1). Shikonin and its derivatives were rapidly degraded with increasing light intensities. Those were degraded by half within 6.7–8.1 h at 10000 lx and within 4.2–5.1 h at 20000 lx light. The half-life values of anthocyanins and betanine under 4390 lx light intensity are 27–34 h (pH 2.5) and 2.5 h (pH 5.0) at 55 °C, respectively (Attoe and von Elbe, 1981).

In conclusion, we isolated five shikonin derivatives from the roots of *L. erythrorhizon* and examined thermal and light stabilities in an aqueous solution. The isolated shikonin derivatives were relatively stable against heat ( $t_{1/2}$  = 23.4–65.6 h, 50 °C) and light ( $t_{1/2}$  = 6.67–8.11 h, 10000 lx) at acidic pH compared with other natural colorants. For example, the thermal degradation half-life values of anthocyanins and betacyanin are 40–44 h (55 °C, pH 2.5) (Attoe and von Elbe, 1981) and 3 h

(50 °C) (Merin et al., 1987), respectively. Anthocyanins and betanine show photodegradation half-life values of 27–34 h (pH 2.5) and 2.5 h (pH 5.0) under 4390 lx light at 55 °C (Attoe and von Elbe, 1981), respectively. Therefore, the shikonin pigments would have competitive physical stability for use as possible value-added colorants for foods and cosmetics.

#### LITERATURE CITED

- Ahn, B.-Z.; Baik, K.-U.; Kweon, G.-R.; Lim, K.; Hwang, B.-D. Acylshikonin Analogues: Synthesis and Inhibition of DNA Topoisomerase-I. *J. Med. Chem.* **1995**, *38*, 1044–1047.
- Attoe, E. L.; von Elbe, J. H. Photochemical Degradation of Betanine and Selected Anthocyanins. *J. Food Sci.* **1981**, *46*, 1934–1937.
- Cho, M.-H.; Yoon, H.-H.; Hahn, T.-R. Thermal Stability of the Major Color Component, Cyanidin 3-glucoside, from a Korean Pigmented Rice Variety in Aqueous Solution. *Agric. Chem. Biotechnol.* **1996**, *39*, 245–248.
- Chung, M.-S.; Lee, M.-S. Stability and Sensory Evaluation of Naphthoquinone Pigments from the Roots of *Lithospermum erythrorhizon*. *Korean J. Food Sci. Technol.* **1994**, *26*, 157–161.
- Chung, M.-S.; Lee, M.-S. Stability of Naphthoquinone Pigments Isolated from the Roots of *Lithospermum erythrorhizon* by Various Temperatures and Metal Ions. *Korean J. Food Sci. Technol.* **1995**, *27*, 97–100.
- Kyogoku, K.; Terayama, H.; Tachi, Y.; Suzuki, T.; Komatsu, M. Constituents of shikon. II. Comparison of contents, constituents, and antibacterial effect of fat soluble fraction between nanshikon and koshikon. *Shoyakugaku Zasshi* **1973**, *27*, 31–36.
- Merin, U.; Gagel, S.; Popel, G.; Bernstein, S.; Rosenthal, I. Thermal Degradation Kinetics of Prickly-Pear-Fruit Red Pigment. *J. Food Sci.* **1987**, *52*, 485–486.
- Mizukami, H.; Konoshima, M.; Tabata, M. Variation in Pigment Production in *Lithospermum erythrorhizon* Callus Cultures. *Phytochemistry* **1978**, *17*, 95–97.
- Morimoto, I.; Hirata, Y. New Naphthoquinone Derivatives from *Lithospermum erythrorhizon*. *Tetrahedron Lett.* **1966**, 3677–3680.
- Morimoto, I.; Kishi, T.; Ikegami, S.; Hirata, Y. Naphthoquinone Derivatives from *Lithospermum erythrorhizon* Siebold et Zuccarint. *Tetrahedron Lett.* **1965**, 4737–4739.
- Tabata, M.; Mizukami, H.; Hiraoka, N.; Konoshima, M. Pigment Formation in Callus Cultures of *Lithospermum erythrorhizon*. *Phytochemistry* **1974**, *13*, 927–932.
- Tanaka, S.; Tajima, M.; Tsukada, M.; Tabata, M. A Comparative Study on Anti-inflammatory Activities of the Enantiomers, Shikonin and Alkannin. *J. Nat. Prod.* **1986**, *49*, 466–469.
- Tsukada, M.; Fukui, H.; Habara, C.; Tabata, M. Comparative studies on naphthoquinone derivatives in various crude drugs of "zicao" (shikon). *Shoyakugaku Zasshi* **1983**, *37*, 299–305.

Received for review March 22, 1999. Revised manuscript received June 14, 1999. Accepted July 6, 1999. This work was supported by grants from the Nondirected Research Fund, Korea Research Foundation (1998), and Advanced R&D Program, Ministry of Agriculture, Forestry, and Fisheries (1998).

JF9902853