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# Propionylshikonin from the Roots of *Lithospermum erythrorhizon*

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A shikonin derivative was isolated from the roots of *Lithospermum erythrorhizon* with silica gel column chromatography and preparative TLC. The structure of the isolated pigment was identified as propionylshikonin by NMR and mass spectrometric analysis. The isolation of propionylshiknin was for the first time in the nature.

**Key words** : *Lithospermum erythrorhizon*, Boraginaceae, Naphthoquinone, Propionylshikonin

## INTRODUCTION

Red pigments in the roots of *Lithospermum erythrorhizon* S. et Z. (Boraginaceae) have been used for antibacterial, antiinflammatory, antipyretic, and antidotal purposes in oriental countries (Tanaka *et al.*, 1986). The red pigments have also been used as raw materials for dyes, cosmetics and food colorants. Previous studies showed that shikonin and its derivatives exhibited antitumor actions and cytotoxic effects against some cell lines (Lee and Ahn, 1986; Kim and Ahn, 1990). Naturally occurring and synthetic shikonins showed inhibitory activity against DNA topoisomerase-I. Especially, acetylshikonin, n-pro-panoylshikonin (propionylshikonin), and 4-pentenoylshiko-nin were strong inhibitors of DNA topoisomerase-I (Ahn *et al.*, 1995).

The color components of the roots of *L. erythrorhizon* are composed of shikonin and its derivatives. So far, 9 shikonin derivatives have been isolated and identified from *L. erythrorhizon*. These are shikonin, deoxyshikonin, acetylshikonin, isobutylshikonin, β,β-dimethylacrylshikonin, isovalerylshikonin, tetracrylshikonin, β-hydroxyisovaleryl-shikonin, and α-methyl-*n*-butylshikonin (Morimoto *et al.*, 1965; Morimoto and Hirata, 1966; Kyogoku *et al.*, 1973; Mizukami *et al.*, 1978; Chung and Lee, 1994; Cho *et al.*, 1999). In the present study, we firstly isolated and identified propionylshikonin from the roots of *L. erythrorhizon* cultivated in Korea.

## MATERIALS AND METHODS

### Materials

The roots of *Lithospermum erythrorhizon* (Boraginaceae) were purchased from the local market and stored at room temperature. Silical gel 60 (0.063~0.2 mm) and TLC plate precoated Kiegel 60, 0.2 mm layer thickness were purchased from Merck Chemical Co.. Solvents includ-ing hexane, ethyl acetate, and ethanol were obtained from Hayman Ltd. and Tedia Co., Inc.

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### Spectrometry

$^1H$ and $^{13}C$ NMR spectra were recorded on a 400 MHz FT-NMR (JEOL) spectrometer with TMS as an internal standard. UV/Vis spectra were measured with a Varian DMS 300 spectrophotometer. IR spectra were recorded using a Jasco FT-IR 430 spectrometer. Mass spectra were obtained using a JEOL JMS-AS505 WA mass spectrometer.

### Extraction and isolation

The sliced roots of *Lithospermun erythrorhizon* (600 g) were extracted with 6 L of hexane for 24 h at room temperature and concentrated with a rotary evaporator. The concentrate was applied to a silica gel column and gradiently eluted with mixtures of hexane and ethyl-acetate (20:1~1:1). The fraction eluted with 6:1 mixture of hexane and ethylacetate was pooled and further isolated with preparative TLC developed with a mixture of hexane and ethylacetate (5:1). A red pigment (7.2 mg) was obtained: mp: 64~66°C. $[\alpha]_D^{24}$: +260 (C 1.0, EtOH). UV (EtOH) $\lambda_{max}$ nm (log ε): 561 (3.65), 521 (3.84), 489 (3.78), 276 (3.89). IR (KBr) $\nu_{max}$ $cm^{-1}$: 2921, 1744, 1614, 1459, 1206, 787. EIMS 70 eV, *m/z* (rel. int): 344 $[M]^+$ (3), 270 $[M\text{-}CH_3CH_2COOH]^+$ (100), 255 (33), 220 (32), 219 (25), 69 (14), 57 (83).

## RESULTS AND DISCUSSION

A red pigment was isolated from the roots of *L.*

*erythrorhizon* with silica gel column chromatography and preparative TLC. The UV/Vis measurement of the isolated pigment in EtOH showed a typical spectrum of shikonin pigments with $\lambda_{max}$ of 561, 521, 489, and 276 nm (Morimoto *et al.*, 1965; Morimoto and Hirata, 1966; Cho *et al.*, 1999). The IR spectrum showed absorption bands at 1744 and 1206 $cm^{-1}$, indicating the presence of an ester group. The chemical structure of the isolated pigment was determined with NMR and EIMS spectrometry. The $^1H$ NMR spectrum showed the existence of shikonin skeleton and propionyl group. Two distinct singlet peaks at δ 12.44 and 12.60 were assigned as phenolic OH due to the fact that shikonin and other shikonin derivatives showed the same pattern. Also these proton signals had no $^{13}C$ peak correlation in the $^1H$-$^{13}C$ COSY spectrum. Two aromatic proton peaks at δ 6.98 (1H) and δ 7.19 (2H) were assigned as H-3 and H-6, 7, respectively. The $^{13}C$ peak of C-1' appeared at δ 69.2 to give an account of oxygen atom. The proton signal at δ 6.03 (1H) correlated with $^{13}C$ peak at δ 69.2 in the $^1H$-$^{13}C$ COSY spectrum was assigned to H-1'. Two proton peaks at δ 2.47 and δ 2.62 coupled with a proton peak at δ 6.03 in $^1H$-$^1H$ COSY spectrum (data not shown) was determined as H-2'. The proton peak at δ 5.12 (2H) correlated with the $^{13}C$ peak at δ 117.7 in the $^1H$-$^{13}C$ COSY spectrum was assigned to H-3'. Two singlet peaks at δ 1.69 (3H) and δ 1.58 (3H) were assigned to H-5' and H-6' by analyzing the $^1H$-$^{13}C$ COSY spectrum, respectively. All of these data were in good agreement with shikonin and other shikonin derivatives isolated from *L. erythrorhizon* in this lab (Cho *et al.*, 1999). The quartet peaks and the triplet peaks at δ 2.43 (2H) and δ 1.18 (3H) were assigned to H-2" and H-3", respectively. Fragment ion peak $[M\text{-}CH_3CH_2COOH]^+$ at 270 *m/z* in the EIMS spectrum indicated that the isolated pigment has a basic skeleton of shikonin pigments. Molecular ion peak $[M]^+$ at 344 *m/z* in the EIMS spectrum also agreed with the molecular formula of propionylshikonin ($C_{19}H_{20}O_6$). Therefore, the structure of the isolated red pigment was identified as propionylshikonin (Fig. 1). Furthermore its structure was assured by the authentic sample synthesized by Ahn *et al* (1995). The $^1H$ and $^{13}C$ NMR data of the isolated pigment were summarized in Table I. The IR and EIMS spectral data, and mp (64~66°C) also agreed well with those of synthetic propionylshikonin synthesized by Ahn *et al* (1995). Japanese workers isolated 9 shikonin derivatives from *L. erythrorhizon* (Morimoto *et al.*, 1965; Morimoto and Hirata, 1966; Kyogoku *et al.*, 1973; Mizukami et al., 1978). All naphthoquinone pigments isolated from the roots of *L. erythrorhizon* were shikonin derivatives (*R*-configuration) (Morimoto *et al.*, 1965; Morimoto and Hirata, 1966; Tanaka *et al.*, 1986). In Korea, five shikonin derivatives were isolated and identified from *L. erythrorhizon* (Chung and Lee, 1994;

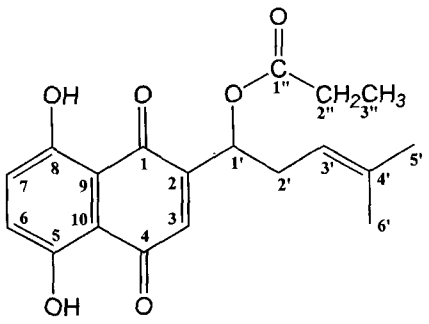


**Fig. 1.** Structure of propionylshikonin isolated from the roots of *Lithospermum erythrorhizon*.

**Table I.** The $^1H$ and $^{13}C$ NMR data of the isolated pigment from the roots of *Lithospermum erythrorhizon* (in $CDCl_3$, chemical shifts in ppm)

| Position | $^1H$ NMR | $^{13}C$ NMR |
|---|---|---|
| 1,4 | | 176.8, 178.3 |
| 2 | | 148.4 |
| 3 | 6.98 (1 H, d, *J* = 1.0 Hz) | 131.4 |
| 5,8 | | 166.8, 167.3 |
| 6,7 | 7.19 (2 H, s) | 132.7, 132.8 |
| 9,10 | | 111.6, 111.8 |
| 1' | 6.03 (1 H, m) | 69.2 |
| 2' | 2.47 (1 H, m), 2.62 (1 H, m) | 32.8 |
| 3' | 5.12 (1 H, m) | 117.7 |
| 4' | | 136.0 |
| 5' | 1.69 (3 H, s) | 25.8 |
| 6' | 1.58 (3 H, s) | 17.9 |
| 1" | | 173.2 |
| 2" | 2.43 (2H, q, *J* = 7.6 Hz) | 27.6 |
| 3" | 1.18 (3 H, t, *J* = 7.6 Hz) | 9.0 |
| OH | 12.44 (1 H, s), 12.60 (1H, s) | |

Cho *et al.*, 1999). However, no data has been documented for the presence of propionylshikonin from natural products. Here, we report a shikonin derivative, propionylshikonin, isolated firstly from the roots of *L. erythrorhizon* cultivated in Korea.

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