Phytochemical Constituents from the Seeds of *Lithospermum erythrorhizon*

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**Abstract** — Phytochemical constituents were isolated from the seeds of *Lithospermum erythrorhizon* through open column chromatography and prep-HPLC. Their structures were identified as β-sitosterol (1), daucosterol (2), luteolin (3), and allantoin (4) on the basis of spectroscopic analysis. Among them, luteolin (3) was isolated for the first time from the plant.

**Keywords** — *Lithospermum erythrorhizon*, Boraginaceae, Column chromatography, Constituent, Luteolin

**Introduction**

*Lithospermum erythrorhizon* (Boraginaceae) is a perennial herbaceous species which is found in mountains and fields in Korea, Japan and China. Red pigments of this plant accumulate in the cork layers of the roots. The plant grows up to 0.7 m. Its floral season is June to September (Tabata, 1996; Cho et al., 1999a; Lee, 2006). It has been used medicinally for wounds (Tou no et al., 2005), burns, and haemorrhoids (Tabata et al., 1974) and also for staining fabrics and food colorants (Lee et al., 2000). It possesses a wide spectrum of biological properties, including anti-tumor, anti-fungus, anti-HIV (Han et al., 2008), anti-inflammatory (Kang et al., 1998), anti-microbial, immunostimulating and contraceptive (Shen et al., 2002).

The color components of *L. erythrorhizon* roots are composed of shikonin and its derivatives, such as deoxyshikonin, acetylshikonin, isobutylshikonin, β,β-dimethylacrylshikonin, isovalerylshikonin, tetracylshikonin, β-hydroxyisovalerylshikonin, α-methyl-n-butylshikonin, and propionylshikonin (Cho et al., 1999b; Morimoto et al., 1965; Zhang et al., 2002). Also, lithospermic and rosmarinic acids (Fukui et al., 1984), and lithosperman B (Yamamoto et al., 2000) have been isolated from *L. erythrorhizon* roots.

Up to now, there have been many reports on the isolation and identification of compounds from *L. erythrorhizon* roots and on their medicinal effects, but no similar reports on compounds from *L. erythrorhizon* seeds. During the course of our continued studies on the compounds from *L. erythrorhizon* seeds, four compounds are isolated. This paper deals with the isolation and structure elucidation of compounds 1-4.

**Experimental**

**Plant material** — The seeds of *Lithospermum erythrorhizon* were collected from Jeongseon (Oct. 2007), Kangwon Province, Korea, and verified by Prof. Young-Hee Ahn, Chung-Ang University, Korea.

**General procedures** — The electron ionization mass spectrometry (EI-MS) was measured with a JEOL JMS-600W (Japan) mass spectrometer. 1H- and 13C-nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AVANCE 300 NMR (Germany) spectrometer in CDCl₃, pyridine or DMSO using TMS as an internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (J) were expressed in hertz. TLC analysis was conducted with Kiesel gel 60 F254 (Art. 5715, Merck Co., Germany) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by spraying with 10% H₂SO₄ followed by charring. Open column chromatography was conducted with silica gel (200-400 mesh ASTM; Merck Co., Germany). All other chemicals and reagents were analytical grade. Recycling preparative HPLC was conducted by a JAI LC-9104 (Japan) system and determination was performed by an L-6050 system pump with UV-3702 system UV/VIS detector.
**Extraction and isolation**—The air-dried whole seeds of *L. erythrhorizon* (4,695 g) were ground into powder and extracted with MeOH (10 liters × 3) under reflux. The resultant extracts were combined and concentrated under reduced pressure to afford 325 g of the residue. The MeOH extract was suspended in water and then fractionated successively with equal volumes of *n*-hexane (230 g), CHCl₃ (9 g), EtOAc (4 g) and *n*-BuOH (23 g). A portion of the CHCl₃ fraction (8.0 g) was chromatographed on a silica gel column eluting with a gradient of *n*-hexane-EtOAc (100% *n*-hexane up to 80% EtOAc) to afford ten subfractions (subfrs. C₁⁻C₁₀). Among them, the subfrs. C₃ and C₁₀ were chromatographed on a silica gel column eluting with a gradient of EtOAc-CHCl₃ (n-hexane up to 100% CHCl₃) and EtOAc-MeOH mixture of increasing polarity) to afford compounds 1 (n-hexane : EtOAc, 4 : 6), respectively. A portion of the EtOAc fraction (3.0 g) was chromatographed on a silica gel column eluting with a gradient of CHCl₃-CHCl₃ (100% CHCl₃) and EtOAc : MeOH : MeOH mixture of increasing polarity) to afford 12 subfractions (subfrs. E₁⁻E₁₂). The subfr. E₁₂ were eluted within 42 min by prep-HPLC using a JAIGEL-GS 12 subfractions (subfrs. E₁⁻E₁₂). The subfr. E₁₂ were (EtOAc : MeOH mixture of increasing polarity) to afford (100% *n*-hexane up to 80% EtOAc) to afford ten subfractions (subfrs. C₁⁻C₁₀). Among them, the subfrs. C₃ and C₁₀ were chromatographed on a silica gel column eluting with a gradient of *n*-hexane-EtOAc to give compounds 1 (n-hexane : EtOAc, 97 : 3) and 2 (n-hexane-EtOAc, 4 : 6), respectively. A portion of the EtOAc fraction (3.0 g) was chromatographed on a silica gel column eluting with a gradient of *n*-hexane-EtOAc (100% *n*-hexane up to 100% EtOAc) and EtOAc : MeOH : MeOH mixture of increasing polarity) to afford 12 subfractions (subfrs. E₁⁻E₁₂). The subfr. E₁₂ were eluted within 42 min by prep-HPLC using a JAIGEL-GS column with UV/VIS detection set at 245 nm. The mobile phase was CHCl₃-MeOH (80 : 20, v/v) at ambient temperature. A prep-HPLC separation of the subfrs. C₉ and E₁₂ led to the isolation of compound 3. A portion of the *n*-BuOH fraction (10.0 g) was chromatographed on a silica gel column eluting with a gradient of CHCl₃ : MeOH (100% CHCl₃ up to 80% MeOH) to afford 11 subfractions (subfrs. B₁⁻B₁₁). The subfr. B₇ was chromatographed on a silica gel column eluting with CHCl₃ : MeOH (6 : 4) to give compound 4.

**Compound 1**: white powder; *R*ᵣ = 0.24 (n-hexane : EtOAc - 8 : 2); EI-MS *m/z*: 414 [M⁺] (100.0), 396 (53.1), 381 (58.3), 329 (83.1), 303 (110.0), 289 (118.8), 273 (20.8), 255 (26.3), 231 (15.8), 213 (23.2), 159 (17.0), 145 (21.4); ¹H-NMR (300 MHz, CDCl₃): δ 3.52 (1H, m, H-3), 2.27 (3H, s, H-18), 0.94 (3H, d, J = 6.3 Hz, H-21), 0.95 (3H, s, H-19), 1.00 (3H, d, J = 6.3 Hz, H-21), 0.94 (3H, d, J = 5.7 Hz, H-26), 0.90 (3H, d, J = 6.9 Hz, H-27), 0.89 (3H, m, H-29), 0.50 (1H, d, J = 7.8 Hz, H-1'); ¹³C-NMR (75 MHz, pyridine): δ 36.8 (C-1), 29.3 (C-2), 78.7 (C-3), 38.3 (C-4), 140.4 (C-5), 121.2 (C-6), 31.4 (C-7), 31.3 (C-8), 49.6 (C-9), 36.2 (C-10), 20.6 (C-11), 40.1 (C-12), 41.8 (C-13), 56.2 (C-14), 23.9 (C-15), 27.8 (C-16), 55.1 (C-17), 11.7 (C-18), 19.1 (C-19), 35.5 (C-20), 18.6 (C-21), 33.3 (C-22), 25.4 (C-23), 45.1 (C-24), 28.7 (C-25), 18.9 (C-26), 17.3 (C-27), 22.6 (C-28), 11.8 (C-29), 100.8 (C-31), 73.5 (C-3'), 72.9 (C-3'), 70.1 (C-4'), 76.7 (C-5'), 61.1 (C-6').

**Compound 2**: white powder; *R*ᵣ = 0.56 (CHCl₃ : MeOH - 8 : 2); EI-MS *m/z*: 286 [M⁺] (100.0), 258 (12.2), 153 (18.5), 134 (6.8), 129 (9.4); ¹H-NMR (300 MHz, DMSO): δ 6.67 (1H, s, H-3), 6.19 (1H, d, J = 2.0 Hz, H-6), 6.44 (1H, d, J = 2.0 Hz, H-8), 7.33 (1H, d, J = 2.1 Hz, H-2), 6.94 (1H, d, J = 8.1 Hz, H-5'), 7.42 (1H, dd, J = 2.1, 8.1 Hz, H-6'), 12.98 (1H, s, 5-OH). ¹³C-NMR (75 MHz, DMSO): δ 163.9 (C-2), 103.7 (C-3), 181.6 (C-4), 161.5 (C-5), 98.8 (C-6), 164.1 (C-7), 93.8 (C-8), 157.3 (C-9), 103.7 (C-10), 119.0 (C-1'), 113.4 (C-2'), 145.7 (C-3'), 149.7 (C-4'), 116.0 (C-5'), 121.5 (C-6').

**Compound 3**: yellow powder; *R*ᵣ = 0.56 (CHCl₃ : MeOH - 45:55); EI-MS *m/z*: 158 [M⁺] (9.8), 141 (10.9), 130 (100.0), 115 (31.4), 87 (80.8), 70 (10.8), 60 (22.4); ¹H-NMR (300 MHz, DMSO): δ 10.56 (1H, s, 1-NH), 8.07 (1H, s, 3-NH), 5.23 (1H, d, J = 8.1 Hz, H-4), 6.90 (1H, d, J = 8.1 Hz, 6-NH), 5.80 (2H, s, 8-NH); ¹³C-NMR (75 MHz, DMSO): δ 156.9 (C-2), 62.5 (C-4), 173.7 (C-5), 157.4 (C-7).

**Results and Discussion**

A chromatographic separation of the MeOH extracts from the seed of *L. erythrhorizon* led to the isolation of compounds 1 - 4 (Fig. 1).

Compound 1 was obtained as white powders from the CHCl₃ fraction and it showed a molecular ion peak at *m/z* 414 [M⁺] in the EI-MS. The ¹H-NMR spectrum of 1 showed existence of sterol skeleton. The two angular methyl singlets of 18- and 19-Me at δ 0.68 and 1.01, and the three doublets of 21-, 26-, and 27-Me at δ 0.80, and the one triplet of 29-Me at δ 0.81 were observed, respectively. The olefinic proton broad doublet one signal at δ 5.35 was showed H-6. The ¹³C-NMR spectrum of 1 showed 27 resonances, and C-5 and -6 signals were noticed at δ 141.1 and 122.2, respectively. Accordingly, the structure of 1 was elucidated as β-