

## Isolation of Hepatoprotective Phenylpropanoid from *Lactuca indica*

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**Abstract** – In continuing our search for biologically active compounds from Korean Compositae medicinal plants, we investigated the constituents of the aerial parts of *Lactuca indica* L. and isolated a phenylpropanoid derivative from its MeOH extract. The chemical structure was characterized by spectroscopic methods, including 1D and 2D NMR to be di-*E*-caffeoyl-*meso*-tartaric acid (**1**). Compound **1** was isolated for the first time from this plant. In this paper, we suggest that the NMR assignment at C-2 of (+)-taraxafolin-B should be corrected. In the human HBV-transfected liver cell line HepG2.2.15, the compound **1** effectively reduced HBV DNA level in the release of mature HBV particles from HepG2.2.15 cultivation.

**Keywords** – *Lactuca indica* L. Compositae, Di-*E*-caffeoyl-*meso*-tartaric acid, Hepatoprotective activity

### Introduction

*Lactuca indica* L. (Compositae) is widely distributed throughout Korea. This indigenous herb is an edible wild vegetable that is traditionally used as a folk medicine for inflammatory, bacterial infection, and intestinal disorders (Kan, 1986). Various compounds including terpenoids (Hui *et al.*, 1971; Fan *et al.*, 2004), sterols (Fan *et al.*, 2004) and flavonoids (Makoto *et al.*, 1978) were isolated from this plant. Several biological activities of this plant have been reported, including antimutagenic activity against indirect-acting mutagens (AFB<sub>1</sub> and B(a)P) (Kusamran *et al.*, 1998) and stimulation of differentiation of the mouse melanoma cell line, B16 2F2 (Hata *et al.*, 2003). In continuing our search for biologically active compounds from Korean Compositae medicinal plants, we investigated the constituents of the aerial parts of *L. indica* and reported terpenoids, phenolic constituents and their hepatoprotective activity (Kim *et al.*, 2007; Kim *et al.*, 2008). We conducted a further chemical investigation of the aerial parts of *L. indica*, which led to isolation of a phenylpropanoid derivative, di-*E*-caffeoyl-*meso*-tartaric acid (**1**). The structure of **1** was elucidated by spectroscopic methods, including 1D and 2D NMR. Compound **1** was isolated for the first time from this plant. Compound **1** was evaluated for hepatoprotective activity by the HBV assay *in vitro*. This paper describes the isolation, structural

elucidation, and hepatoprotective activity of **1**.

### Experimental

**General** – Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 Polarimeter. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded with a Shimadzu UV-1601 UV-Visible spectrophotometer. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer. LC-ESI/MS data on an Agilent 1100LC/MSD trap SL LC/MS. Preparative HPLC was performed using a Gilson 306 pump with a Shodex refractive index detector and Econosil<sup>®</sup> RP-18 10  $\mu$  column (250  $\times$  10 mm). Silica gel 60 (Merck Co., Germany, 70–230 mesh, and 230–400 mesh) and RP-C<sub>18</sub> silica gel (Merck Co., Germany, 230 - 400 mesh) were used for column chromatography. TLC was performed using Merck precoated Silica gel F<sub>254</sub> plates and RP-18 F<sub>254s</sub> plates. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.).

**Plant materials** – The aerial parts of *L. indica* were collected in Suwon, Korea, in May 2005, and the plant was identified by one of the authors (Y.H.K.). A voucher specimen (SKKU-2005-05) of the plant was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

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**Extraction and isolation** – The aerial parts of *L. indica* (5 kg) were extracted at room temperature with 80% MeOH and evaporated under reduced pressure to give a residue (200 g), which was dissolved in water (800 mL) and solvent-partitioned (*n*-hexane, CHCl<sub>3</sub> and *n*-BuOH) to give *n*-hexane (20 g), CHCl<sub>3</sub> (12 g), and *n*-BuOH (75 g) soluble extracts. The *n*-BuOH fraction (45 g) was separated over a silica gel column with a solvent system of CHCl<sub>3</sub> : MeOH : H<sub>2</sub>O (30 : 10 : 1 - 13 : 7 : 1) as the eluent to give six fractions (B1-B6). Fraction B6 (3.0 g) was separated over an RP-C<sub>18</sub> silica gel column with 30% MeOH as the eluent to give six subfractions (B61-B66). Subfraction B62 (350 mg) was applied to column chromatography over Sephadex LH-20 (Pharmacia Co.), eluting with a solvent system of MeOH : H<sub>2</sub>O (4 : 1) and purified further by semi-preparative HPLC, using aqueous 28% MeOH over 30 min at a flow rate of 2.0 mL/min (Econosil® RP-18 10 μ column; Shodex refractive index detector) to afford **1** (25 mg, *R*<sub>f</sub> = 13.5 min).

**Di-*E*-caffeoyl-meso-tartaric acid (1)** Yellowish powder, mp: 120 - 125 °C;  $[\alpha]_D^{25}$  : +50.6° (c 0.51, MeOH); IR  $\nu_{\max}$  cm<sup>-1</sup>: 3392, 2713, 1701, 1601, 1525, 1385, and 1157; UV  $\lambda_{\max}$  (MeOH) nm (log  $\epsilon$ ): 217 (2.8), 235 (2.3), 243 (2.3), 299 (2.8), and 325 (3.0); ESI-MS *m/z*: 497 [M + Na]<sup>+</sup>; <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 500 MHz):  $\delta$  7.62 (2H, d, *J* = 16.0 Hz, H-7', H-7''), 7.08 (2H, d, *J* = 1.7 Hz, H-2', H-2''), 6.92 (2H, dd, *J* = 8.1, 1.7 Hz, H-6', H-6''), 6.79 (2H, d, *J* = 8.1 Hz, H-5', H-5''), 6.43 (2H, d, *J* = 16.0 Hz, H-8', H-8''), 5.74 (2H, s, H-2, H-3); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz):  $\delta$  173.3 (C-1, C-4), 167.9 (C-9', C-9''), 148.1 (C-4', C-4''), 145.7 (C-7', C-7''), 145.5 (C-3', C-3''), 126.9 (C-1', C-1''), 121.7 (C-6', C-6''), 115.3 (C-5', C-5''), 114.6 (C-2', C-2''), 114.0 (C-8', C-8''), 75.3 (C-2, C-3); <sup>1</sup>H (D<sub>2</sub>O, 500 MHz) and <sup>13</sup>C (D<sub>2</sub>O, 125 MHz) NMR data, see Table 1.

**The HBV assay *in vitro*** – The hepatoprotective activity of the compound **1** was investigated using the HBV assay *in vitro* (Kim *et al.*, 2007). Cultures of HepG2.2.15 cells grown in DMEM media were supplemented with purified compound **1** from plant extracts. On days 4 of treatment the presence of released HBV particles in the culture media (10 μL) was determined by PCR techniques and compared to untreated controls, positive control with 1-deoxynojirimycin (You *et al.*, 2003), and the compound **1**. Intracellular HBV-specific RNAs were extracted from the harvested HepG2.2.15 cells and could be also determined by RT-PCR techniques (Yang *et al.*, 2005). To monitor the inhibition of HBV secretion from HepG2.2.15 cells, the target region of the HBV surface antigen was amplified in a PCR or RT-PCR reaction. The following set of primers

was employed to amplify the HBsAg sequences: forward primer, 5'-TGC CTC ATC TTC TTG TTG GTT CT-3'; backward primer, 5'- CCC CAA TAC CAC ATC ATC CAT ATA-3' amplifies a 336 nt length of DNA products. The amplified DNA fragments at the HBsAg sequences were then revealed by conventional agarose gel electrophoresis. In order to identify intracellular HBV-specific RNA expression in the HepG2.2.15 cells during treatment, the same target region of HBV surface antigen was amplified in a RT-PCR reaction with the primer sets used above.

## Results and Discussion

Compound **1** was obtained as a yellowish powder. The ESI-MS (*m/z* 497 [M + Na]<sup>+</sup>) and <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data of **1** gave a molecular formula of C<sub>22</sub>H<sub>18</sub>O<sub>12</sub>. The UV spectrum exhibited absorption maxima at 243 and 325 nm, suggesting the presence of aromatic ring in the molecule. The IR spectrum showed absorption bands for hydroxyls (3392 cm<sup>-1</sup>),  $\alpha,\beta$ -unsaturated carbonyl (1701 cm<sup>-1</sup>), and aromatic (1601 and 1525 cm<sup>-1</sup>) functionalities. The <sup>1</sup>H-NMR spectrum showed three aromatic proton signals at  $\delta$  7.08 (d, *J* = 1.7 Hz), 6.92 (dd, *J* = 8.1, 1.7 Hz), and 6.79 (d, *J* = 8.1 Hz), two olefinic proton signals at  $\delta$  7.62 (d, *J* = 16.0 Hz) and 6.43 (d, *J* = 16.0 Hz). The <sup>13</sup>C-NMR spectrum demonstrated the presence of six aromatic carbon signals at  $\delta$  148.1, 145.5, 126.9, 121.7, 115.3, and 114.6, two olefinic carbon signals at  $\delta$  145.7 and 114.0 and a carbonyl carbon signal at  $\delta$  167.9, which implied the presence of a *trans*-caffeoyl moiety from the characteristic shifts and coupling constant of signals at  $\delta$  7.62 (*J* = 16.0 Hz) and 6.43 (*J* = 16.0 Hz). In addition, there was an oxygenated proton signal at  $\delta$  5.74 (s) in the <sup>1</sup>H-NMR spectrum. The HMQC spectrum revealed that the proton at  $\delta$  5.74 is attached to the carbon signal at C-2/C-3 ( $\delta$  75.3). The carbon signal at  $\delta$  75.3 and a carboxyl carbon signal at  $\delta$  173.3 observed in the <sup>13</sup>C-NMR spectrum were characteristic shifts of the tartaric acid (Bergmana *et al.*, 2001). The downfield shift of H-2/H-3 compared to tartaric acid showed that the caffeoyl moiety was attached at the hydroxyl function of the tartaric acid (Bergmana *et al.*, 2001). This was also supported by the HMBC spectrum, which showed that H-2/H-3 were correlated to C-1/C-4 ( $\delta$  173.3) and C-9'/C-9'' ( $\delta$  167.9). Although a caffeoyl moiety was assigned in the <sup>1</sup>H- and <sup>13</sup>C-NMR signals, the molecular ion peak [M + Na]<sup>+</sup> at *m/z* 497 indicated the presence of a symmetrical dicaffeoyl-tartaric acid. The relative configuration of tartarate residue of **1** was established as *meso*-tartaric acid, based

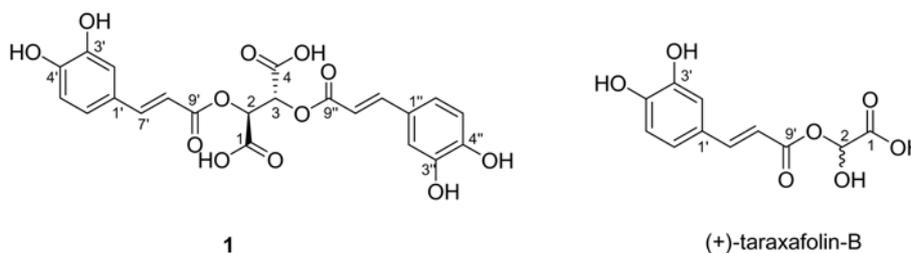
**Table 1**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data for compound **1** from *L. indica* and (+)-taraxafolin-B from *T. formosanum* in  $\text{D}_2\text{O}$ 

Position	<b>1</b> <sup>a</sup>		(+)taraxafolin-B <sup>b</sup>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1,4		173.7		173.9
2,3	5.50 (s)	74.9	5.49 (s)	75.1
1',1''		127.2		127.4
2',2''	6.97 (d, 1.6)	115.3	7.11 (d, 1.6)	115.6
3',3''		144.3		144.6
4',4''		147.2		147.5
5',5''	6.76 (d, 8.0)	116.3	6.84 (d, 8.0)	116.6
6',6''	6.91 (dd, 8.0, 1.6)	123.1	7.04 (dd, 8.0, 1.6)	123.3
7',7''	7.50 (d, 16.0)	146.9	7.60 (d, 16.0)	147.1
8',8''	6.28 (d, 16.0)	114.1	6.37 (d, 16.0)	114.5
9',9''		168.8		169.0

<sup>a</sup>  $^1\text{H}$  (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz) in  $\text{D}_2\text{O}$  ( $\delta$  in ppm)

<sup>b</sup>  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) in  $\text{D}_2\text{O}$  ( $\delta$  in ppm)

Well-resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses.

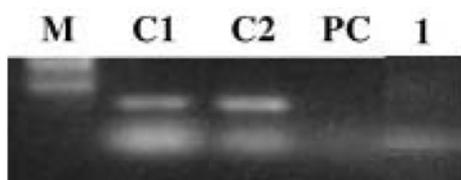
**Fig. 1.** The structures of **1** from *L. indica* and (+)-taraxafolin-B.

on the value of  $^3J_{2,3}$  (Bergmana *et al.*, 2001) and comparison with published  $^{13}\text{C}$ -NMR data (Veit *et al.*, 1991). Therefore, based on all the above evidence, the structure of **1** was assigned as di-*E*-caffeoyl-*meso*-tartaric acid.

According to the survey of literature, the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of (+)-taraxafolin-B, which was reported by Yann *et al.* were very similar to those of the compound **1** (Yann *et al.*, 2005). The spectral data of **1**, particularly  $^{13}\text{C}$ -NMR data, were completely matched with those of (+)-taraxafolin-B isolated from *T. formosanum* (Table 1). We suggest that the assignment at C-2 of (+)-taraxafolin-B should be corrected since its carbon signal at C-2 ( $\delta$  75.1) implies the presence of only one functional group responsible for such a deshielding effect. In the structure of (+)-taraxafolin-B, the carbon signal at C-2 should appear at  $\delta$  95~105, similar to the anomeric carbon of sugar (Stephen *et al.*, 1977) or the carbon of methylenedioxy group (Kim *et al.*, 2010).

The hepatoprotective activity of the isolated compound **1** was assessed using an *in vitro* HBV assay. The

HepG2.2.15 cell line used in this experiment constitutively expresses HBV via an integrated HBV genome and is used extensively for drug evaluation (Korba *et al.*, 1992). After compound **1** was added to the culture media and incubated for 4 days, 10  $\mu\text{L}$  media was used for HBV DNA amplification by PCR. The release of virus particles or the expression of HBV-specific RNAs was positively inhibited by compound **1** at micromolar concentrations after 4 days of treatment. No amplified DNA or a very weak DNA band at the position of 336 bp after agarose gel electrophoresis indicate a positive result, that is, inhibition of virus production. No inhibitory effect on virus production or viral gene expression may result in a unique DNA band at the 336 bp position of the agarose gel. The lack of amplification of viral DNA means that a specific inhibitory molecule had interrupted the processes of viral DNA replication or transcription. From analysis of this PCR products on the agarose gel electrophoresis, we found that compound **1** showed stronger antiviral activity than that of the control molecule of 1-deoxyojirimycin (You *et al.*, 2003), that is, significant



**Fig. 2.** Compound **1** isolated from the *n*-BuOH soluble fraction was assayed for amplification of 336 bp length of HBsAg DNA fragment. 0.39 mM of **1** was added and tested by PCR. **M** lane: 1 Kb size marker; **C1** and **C2** lanes: untreated mock and butanol treated mock as control, respectively. **PC** lane is treated with 1-deoxyojirimycin as a positive control for HBV inhibition.

hepatoprotective activity against hepatitis B virus replication, as shown in Fig. 2.

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