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Distribution of (-)-Yatein in Cupressaceae Family Analysed by High Performance Liquid Chromatography

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The method for the chiral analysis of (-)-yatein was developed and the distribution of this component in the plants of three genera like *Juniperus, Thuja* and *Chamaecyparis* belonging to Cupressaceae family was examined. The chiral analysis of (-)-yatein from the plants was carried out by high performance liquid chromatography on (*R,R*)-Whelk-O1 column using 81 v/v% methanol as mobile phase. The yatein content in the leaves of *Juniperus* was the highest in compare with that of the other two genera, providing the possibility of the chemical discrimination of the plants in *Juniperus* from the other plants in the Cupressaceae family. In general, the yatein content in the leaves was much higher than that in the twigs. This method could be applied for the quality control of (-)-yatein in the plants belonging to Cupressaceae family.

Key words: Yatein, Chiral separation, Cupressaceae family, HPLC

INTRODUCTION

The plants belonging to Cupressaceae family are perennial shrubs used for a treatment for neuralgia, diuretic and aphrodisiac in traditional folk medicine in Korea, China and Japan (Perry, 1980). Several types of extracts from these plants have been found to possess some bioactivities such as antimicrobial (Digrak *et al.*, 1999), antitumor (Ali *et al.*, 1996), radical scavenger (Burits *et al.*, 2001), analgesic (Moreno *et al.*, 1998), anti-inflammatory (Moreno *et al.*, 1998), antiplatelet aggregation (Teng *et al.*, 1994), vasorelaxing (Teng *et al.*, 1994) and antiviral (San Feliciano *et al.*, 1993; Gerhauser *et al.*, 1992) effects.

Chemical investigation of the plants belonging Cupressaceae family has shown the presence of several compounds classified as flavons, lignans and terpenes (Lee *et al.*, 2001; Topcu *et al.*, 1999; Cairnes *et al.*, 1980). (-)-Yatein (Fig. 1) is a lignan of the dibenzylbutyrolactone type, that has been isolated from the plants of Cupressaceae family (Miyata *et al.*, 1998; Chang *et al.*, 2000). It has been recognized as a biosynthetic precursor of deoxypodophyllotoxin and podophyllotoxin, which are well known for their anticancer properties. It was reported that (-)-yatein, isolated from the plants, showed different antitumor activities from the synthetic racemic yatein (Medarde *et al.*, 1995). Hence, stereoselective synthetic methods have been developed to synthesize optically pure yatein and some related compounds (Neidigh *et al.*, 1994; Honda *et al.*, 1994). *Juniperus, Thuja* and *Chamaecyparis* genera belong to the Cupressaceae family and they are similar to each other in the appearance (Lee, 1989). For the view point of the effective utilization of natural resources, it is necessary to analyze the yatein stereospecifically in the plant samples and synthetic materials.

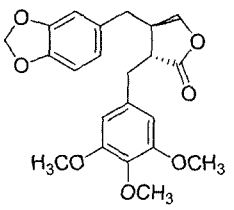

Fig. 1. Structure of (-)-yatein, (3*R*,4*R*)-4-(1,3-benzodioxol-5-ylmethyl) dihydro-3-[(3,4,5-trimethoxyphenyl)methyl]-2(3*H*)-furanone

To date, several reports have been published, concerning

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the achiral or chiral analysis of yatein and related components through lignan profiling by GC-MS (Koulman *et al.*, 2001) or qualitative and quantitative determination by HPLC (Cairnes *et al.*, 1981; Kawai *et al.*, 1999) in plant samples, though precise information about the enantiomeric contents of yatein in various plant species is unavailable. Capillary electrophoresis has been also used for the determination of (-)-yatein in the plants (Lim *et al.*, 2002). In this work, the concentration and distribution of (-)-yatein in the plants of Cupressaceae family was analyzed by means of HPLC using chiral stationary phase.

MATERIALS AND METHODS

Instruments and chemicals

The HPLC system was consisted of SCL-10A system controller, LC-10AD pump, SPD-10MVP diode array detector and CTO-10AS column temperature controller (Shimadzu, Japan). The OR-1590 Chiral Detector (Jasco, Japan) was connected to HPLC for the on-line monitoring of optical rotation of separated peaks. All chemicals and solvents used were of analytical-reagent or HPLC grade. Racemic yatein was synthesized in the laboratory of organic synthesis in Chungnam National University (CNU), Korea, by the published method (Kim *et al.*, 2002). The purity of synthesized yatein measured by HPLC was more than 99%. (-)-Yatein was obtained by collecting the HPLC effluents of synthesized racemic yatein separated on (R,R)-Whelk-O1 column (4.6×250 mm, 5 μm, Regis Tech., IL, USA) with 81 v/v% methanol. The enantiomeric excess of (-)-yatein was more than 98%. (-)-Yatein, measured; $[\alpha]_{589}^{25}$ 33.3° (c = 0.1, $CHCl_3$), reported; $[\alpha]_{546}^{23}$ 24.7° (c = 1.24, $CHCl_3$) by McDoniel *et al.* (1972).

Plant materials and sample preparation

The plant materials, such as *Juniperus chinensis* (CNU-S01), *J. chinensis* var. *globusa* (CNU-S02), *J. chinensis* var. *sargentii* (CNU-S03), *J. chinensis* var. *kaizuka* (CNU-S04), *Thuja occidentalis* (CNU-S05), *Chamaecyparis obtusa* (CNU-S06), *C. pisifera* (CNU-S07) and *C. pisifera* var. *filifera* (CNU-S08) were collected in the campus of CNU in December, 2001. They were identified by Prof. KiHwan Bae in the College of Pharmacy, CNU. The collected samples were separated in leaves and twigs and stored at a cool and dark place. The voucher specimens were deposited at the herbarium in the College of Pharmacy, CNU.

The air dried and powdered sample (0.5 g) was extracted twice with 25 mL of methanol under relfux for 1 h. After filtered, the methanol extract was evaporated under vacuum and the residue was dissolved with 1 mL of 50 v/v% methanol-water. To remove the unwanted components, solid phase extraction was performed with Extract-Clean C18 Column (500 mg/4 mL, Altech, IL, USA) fitted into a Visiprep (Supelco, CA, USA) vacuum manifold. Each column was activated with 5 mL of methanol and the same volume of water. Concentrated sample 0.2 mL was drawn through the column and each column was washed with 3 mL of 10 v/v% methanol-water. Elution was carried out adding 3 mL of 80 v/v% methanol-water to the column. The eluate was evaporated under vacuum and the residue was dissolved in 1 mL of 50 v/v% methanol-water. The solution was directly injected into HPLC or diluted appropriately when the concentration of yatein was higher than upper limit of calibration (0.5 mg/g).

Calibration and sample analysis

For calibration, the twig extract of *C. pisifera*, which contains the least detectable amount of yatein, was spiked with racemic yatein (0.05 to 0.25 mg/mL) and analyzed by HPLC on (R,R)-Whelk-O1 column with 81 v/v% methanol. Linear regression was used for calibration in concentration vs. peak height. For the recovery test, known amounts of racemic yatein standard were spiked to the twigs of *C. pisifera* and extracted under reflux.

RESULTS AND DISCUSSION

Chiral separation of yatein

To find out the optimum mobile phase for the separation of (+)- and (-)-yatein, the mixtures of methanol/water or acetonitrile/water in various ratio of volume were used on the (*R,R*)-Whelk-O1 column (Fig. 2). From the experiments, it was found that methanol was better than acetonitrile for the resolution of yatein enantiomers. As shown in Fig. 2b, a good resolution was achieved on 80 v/v% methanol in water. Further experimental trials revealed 81 v/v% methanol as the optimum mobile phase. The (+)- and (-)-yatein enantiomers were identified by separated injection of each standard. The identification of enantiomers was also carried out by monitoring the optical rotation of HPLC effluent with chiral detector, indicating that the (-)-enantiomer eluted later than (+)-enantiomer (Fig. 3). The chromatogram of racemic yatein showed a good resolution between (+)- and (-)-enantiomer (Fig. 4a). As shown in Fig. 4, only (-)-yatein was found from the extract of *J. chinensis* and no peak related to yatein was found from the twig extract of *C. pisifera*.

Recovery test and calibration

Calibration curves obtained by plotting concentration (mg/mL) vs. peak height (mAU) showed good linearity over the range of 0.025 to 0.5 mg/mL for (-)-yatein, with an R^2 value of 0.9998. Recovery efficiency in the HPLC analysis was more than 96% as shown in Table I. The recovery of (-)-yatein from leaves was somewhat lower

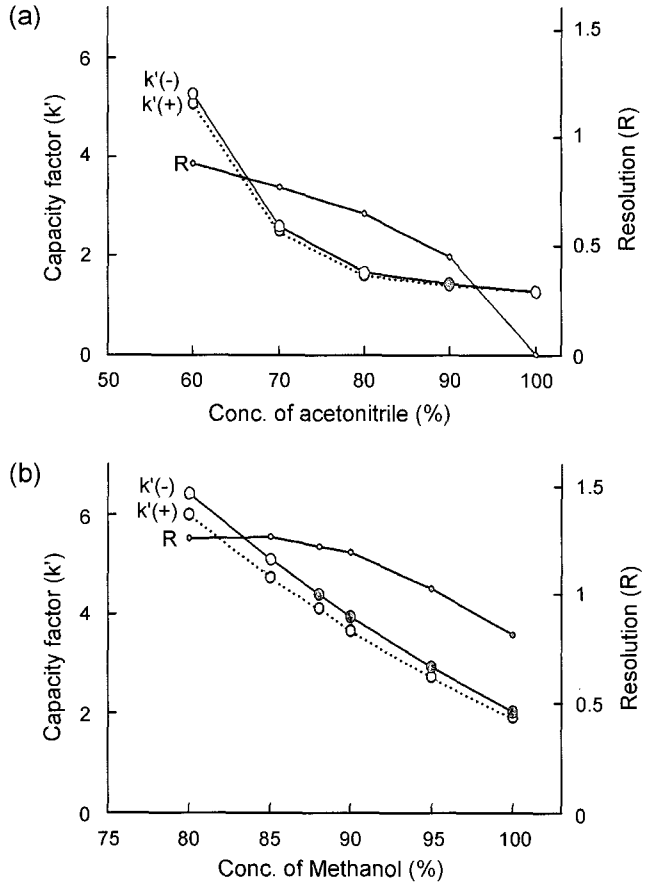

Fig. 2. The effect of acetonitrile and methanol concentration on the retention and resolution of yatein enantiomers. Stationary phase; (*R,R*)-Whelk-O1 column, mobile phase; various concentration of acetonitrile or methanol in water. Capacity factor (*k'*) and resolution (*R*) were calculated as follows; $R = 2(t_{R_1} - t_{R_2})/(W_1 + W_2)$ and $k' = (t_R - t_o)/t_o$, where t_R is retention time of yatein, t_o retention time of solvent and *W* peak width.

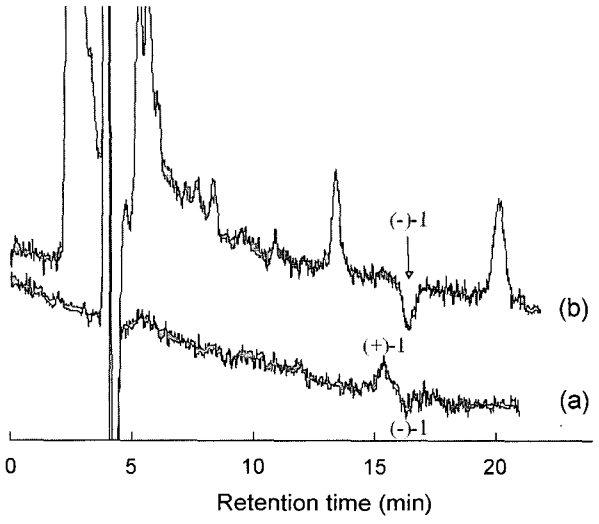

Fig. 3. Chiral chromatogram of yatein enantiomers (a) and an extract of *J. chinensis* sparated on (*R,R*)-Whelk-O1 column with 81 v/v% methanol. Peak 1; yatein, detector; OR-1590 Chiral Detector.

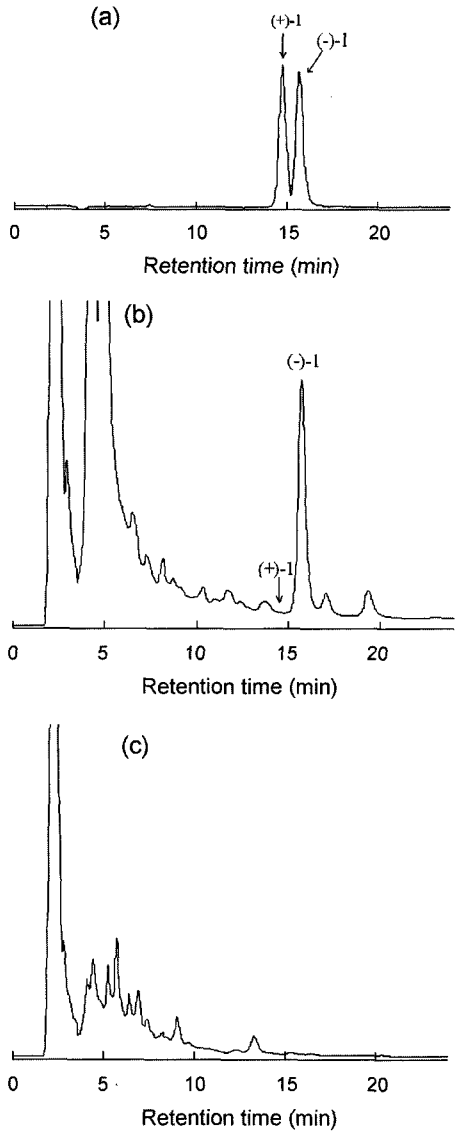

Fig. 4. Chromatograms of (a) racemic yatein standard, an extract of (b) leaves of *J. chinensis* and (c) twigs of *C. pisifera* on (R,R)-Whelk-O1 column with 81 v/v% methanol. Peak 1; yatein, detection; UV 280 nm, flow rate; 1 mL/min.

than that from twigs. Both accuracy and precision criteria indicated that this method was suitable and applicable for the determination of yatein from Cupressaceae plants by HPLC.

Analysis of yatein from plant samples

The concentration of yatein in the plant of Cupressaceae family was determined using the developed method. The chiral separation provided that only (-)-yatein existed in the plant samples. The concentration of yatein in the plants was presented in Table II. *J. chinensis* showed the highest yatein content among the tested plants. The average yatein content of the plants in *Juniperus* genus in

Table I. The recovery of (-)-yatein from *C. pisifera*

Added[a] (mg)	Found[b] (%)	
	Leaves	Twigs
0.5	94.5±3.7	95.8±6.5
1.0	95.8±2.5	96.2±4.3
2.0	95.4±3.2	98.9±4.8

[a] (-)-yatein was added to the twigs of *C. pisifera* (0.5 g).
[b] Data are given as mean±S.D. for three experiments.

Table II. The (-)-yatein content[a] in the plants of Cupressaceae family

Plants	Leaves	Twigs
J. chinensis	11.38	0.64
J. chinensis var. *globusa*	10.20	0.27
J. chinensis var. *sargentii*	8.06	0.50
J. chinensis var. *kaizuka*	2.75	0.14
T. occidentalis	0.27	0.01
C. obtusa	0.34	0.03
C. pisifera	0.03	0.01
C. pisifera var. filifera	0.05	0.02

[a] *Data are represented in mg/g of dried plant sample.*

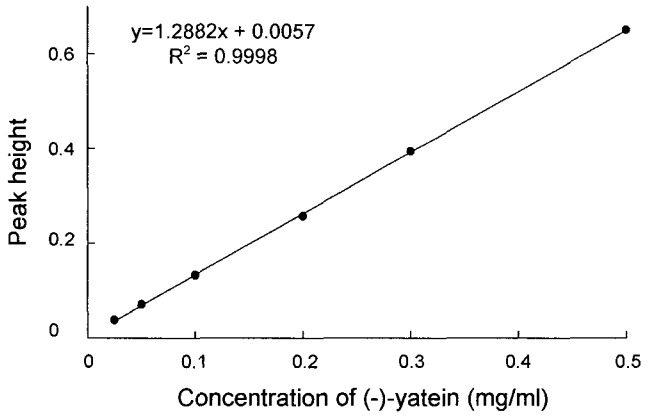

Fig. 5. Calibration of (-)-yatein over the range of 0.025 to 0.5 mg/mL.

the leaves was 8.1 mg/g, about 48 times higher than the average content in the other genera (0.17 mg/g). These facts could be used for the chemical discrimination of the plants in *Juniperus* genus from the other plants in the Cupressaceae family, as the classification of these genus by means of their appearance is not so easy. However, because of the difficulty in homogeneous sampling for the twigs, the precision of the yatein content in twig specimen was relatively high (data not shown). Generally, the yatein content of the leaves was higher than that of twigs. The leaves of *Juniperus* contained about 21 times higher amount of yatein than that found in the twigs. Together, *Juniperus* genus would be more useful than the other genera in Cupressaceae family for the source of yatein related substances.

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