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Short Communication

Identification and determination of honokiol and magnolol from *Magnolia officinalis* by high-performance liquid chromatography with photodiode-array UV detection

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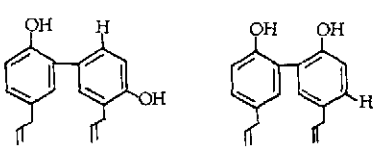
ABSTRACT

An improved high-performance liquid chromatographic technique with photodiode-array detection was developed for the identification and determination of the active principles of *Magnolia officinalis* extracts such as honokiol and magnolol. A reversed-phase column (Nucleosil $7C_{18}$) was eluted with a isocratic system of acetonitrile–0.1% phosphoric acid (65:35). It was found that 19.13 ± 0.62 mg of honokiol and 75.24 ± 3.48 mg of magnolol were contained in the methanol extracts of 1 g of *Magnolia officinalis*.

INTRODUCTION

The root and stem bark of *Magnolia officinalis* (Chinese name: houpo) has been used as a folk medicine in China for the treatment of thrombotic stroke, typhoid fever and headache [1]. Two isomers of neolignans, honokiol and magnolol, have been isolated from the bark of this plant and other *Magnoliaceae* [1]. These two compounds possess antimicrobial activities against Gram-positive and acid-fast bacteria and fungi [2] and central depressant effects [3]. Recent studies indicated that magnolol inhibits intracellular calcium mobilization in platelets caused by collagen, even in the presence of indomethacin [4], and relaxes vascular smooth muscle by releasing endothelium-derived relaxing factor and by inhibiting calcium influx through voltage-gated calcium channels [5].

The determination of honokiol and magnolol by



Honokiol **Magnolol**

Fig. 1. Structures of honokiol and magnolol.

high-performance liquid chromatography (HPLC) [6] and of magnolol and its metabolites by liquid chromatography–mass spectrometry [7–9] have been described. However, none of the methods permits the ultraviolet spectral identification of honokiol and magnolol from *Magnolia officinalis*. In this work, we developed a simple method displaying three-dimensional patterns for the identification and to check the purity of honokiol and magnolol.

EXPERIMENTAL

Materials and reagents

Magnolia officinalis was purchased from a traditional Chinese herbal drug store in Taipei. Authentic compounds, honokiol and magnolol (Fig. 1), were obtained from Nacalai Tesque (Kyoto, Japan) and acetonitrile, methanol, *n*-hexane, ethanol (99.5%) and phosphoric acid (70%) from E. Merck (Darmstadt, Germany).

Apparatus

The HPLC system consisted of a Rheodyne (Cotati, CA, USA) Model 7125 injector, a Waters (Milford, MA, USA) Model 990 photodiode-array detector, which permits the scanning of chromatographic and spectral data, and two Waters Model 510 chromatographic pumps. Separation was achieved on a reversed-phase Nucleosil $7C_{18}$ (particle size 7 μm) column (250 × 4 mm I.D.) (Macherey–Nagel, Düren, Germany) fitted with a column inlet filter (3 mm × 0.5 μm) (Rheodyne) at room temperature. The mobile phase was acetonitrile–water–phosphoric acid (65:35:0.1, v/v/v) of pH 2.4–2.7 at a flow-rate of 1.0 ml/min. The detection wavelengths were 209 nm for honokiol and 218 nm for magnolol.

Extraction

Magnolia officinalis powder (0.5 g) was boiled with 50 ml of extraction solvent [water, methanol, ethanol (99.5%), ethanol (50%), *n*-hexane, 0.1 *M* HCl or 0.1 *M* NaOH] for 15 min. This procedure was repeated twice. The two filtrates were combined and diluted to 100 ml in a volumetric flask.

Authentic samples

The compounds separated by the proposed

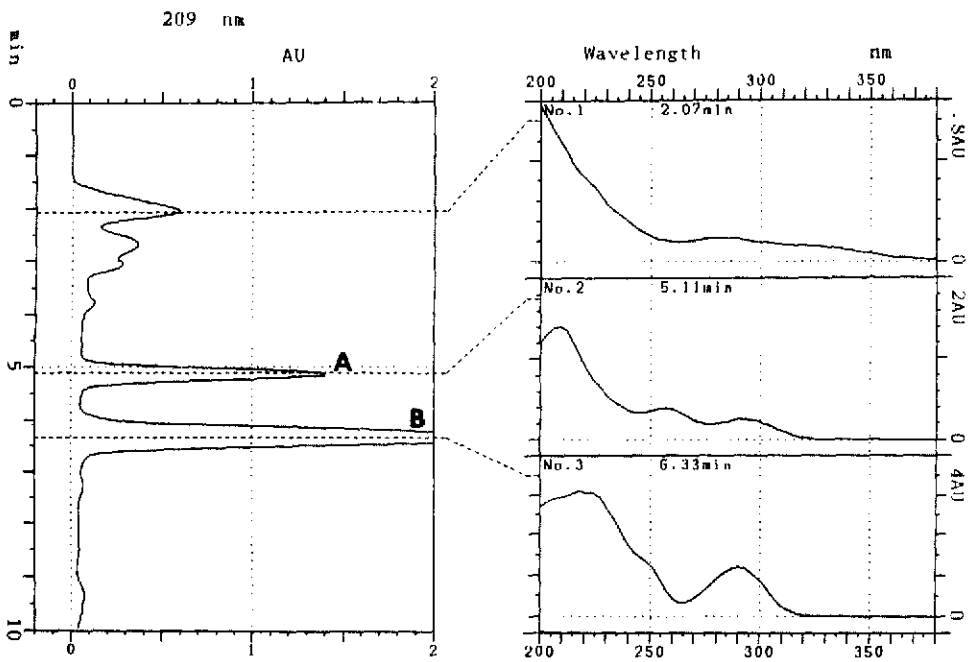


Fig. 2. Chromatogram and UV spectra of ethanol (99.5%) extract of *Magnolia officinalis*. A = honokiol (3.89 μg); B = magnolol (16.18 μg).

HPLC method were identified by comparison of their retention times and spectra with those of authentic samples of honokiol and magnolol.

Determination of honokiol and magnolol

Calibration graphs for honokiol and magnolol dissolved in methanol were constructed by HPLC of various known amounts of these compounds (0.25, 0.5, 1 and 2 μg). The contents of honokiol and magnolol in the crude extract of *Magnolia officinalis* were determined from the regression equations for the lines constructed for the two compounds.

RESULTS AND DISCUSSSION

Under the above conditions, the retention times for honokiol and magnolol were 5.11 and 6.33 min, respectively. Fig. 2 shows the chromatogram and UV spectra of an ethanol (99.5%) extract of *Magnolia officinalis*. The peaks corresponding to honokiol and magnolol were confirmed by the retention times and the UV spectra obtained with photodiode-array detection. Fig. 3 illustrates a three-dimensional chromatogram where both honokiol and magnolol are present. This plot was very useful in the identification of each compound because it allowed the observation of the full UV absorption spectrum of each peak as it eluted from the chromatographic column [10]. Hence the detection of other compounds was easily noted, and co-eluting components could be observed.

The content of each compound in the crude herbal extract was determined from the linear regression equation of the calibration graph for each compound. The equations for honokiol and magnolol were $y = 0.0862x + 0.0028$ ($r = 0.999$) and $y = 0.0655x + 0.0057$ ($r = 0.999$), respectively, where x is amount of compound and y is peak-area response. The linearity range was between 5 ng and 2 μg. The detection limits for honokiol and magnolol, at a signal-to-noise ratio of 4, were 2 and 1 ng, respectively.

Table I gives the contents of honokiol and mag-

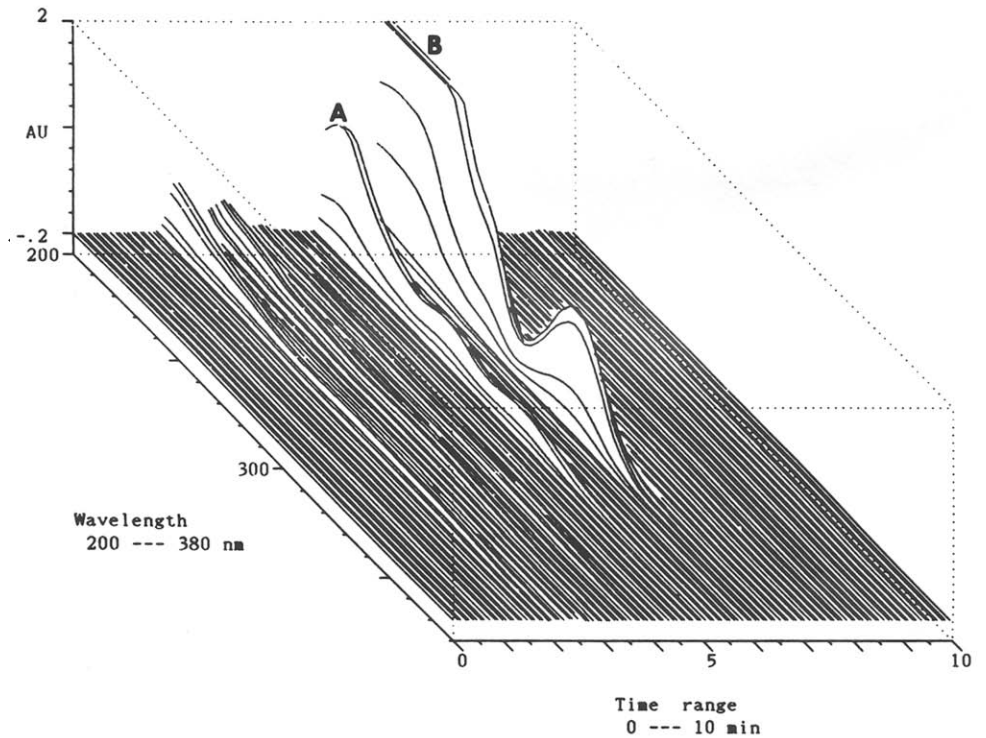


Fig. 3. Three-dimensional chromatogram of ethanol (99.5%) extract of *Magnolia officinalis*. x-Axis is retention time, y-axis absorbance and z-axis UV wavelength. A = honokiol; B = magnolol.

TABLE I

CONTENTS OF HONOKIOL AND MAGNOLOL IN DIFFERENT EXTRACTS OF 1 g OF *MAGNOLIA OFFICINALIS*

Results are means ± S.D. (n=4).

Extraction solvent	Honokiol (mg/g)	Magnolol (mg/g)
Water	0.59 ± 0.09	2.29 ± 0.89
Methanol	19.13 ± 0.62	75.24 ± 3.48
Ethanol (99.5%)	18.88 ± 0.98	83.62 ± 5.23
Ethanol (50%)	17.27 ± 0.21	77.67 ± 0.69
n-Hexane	22.11 ± 1.84	89.87 ± 3.27
0.1 *M* HCl	0.57 ± 0.12	1.70 ± 0.73
0.1 *M* NaOH	18.29 ± 0.27	84.01 ± 1.73

nolol in extracts of *Magnolia officinalis* obtained with different solvents. It appears that n-hexane is the best and 0.1 *M* HCl the worst solvent for the extraction of honokiol and magnolol.

In conclusion, the proposed technique should be useful for the quality control of *Magnolia officinalis*, for stability testing and for pharmacokinetic studies of honokiol and magnolol.

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