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Secondary metabolites from the root of *Lindera reflexa* Hemsl

Suiqing Chen, Lili Wang *, Wanqing Zhang, Yalei Wei

School of Pharmacy, Henan University of Traditional Chinese Medicine, BoXue Road, Jinshui District, ZhengZhou, 450046, China

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ABSTRACT

Fourteen compounds were isolated from the root of *Lindera reflexa* Hemsl. Among these compounds, reflexan A (compound 1) was discovered to possess a novel skeleton and two stilbenes (compounds 2 and 3) were newly discovered compounds. Eleven known compounds (4–14) were also isolated, which included three alkaloids, one coumarin, four stilbenes, two flavonoids and katsumadain. The unusual structure of compounds 1, 2 and 3, along with their absolute configurations, was determined from UV, IR, HR ESI-MS, and 1D and 2D NMR data and by the comparison of experimental and calculated electronic circular dichroism (ECD) spectra. All of the isolates were tested with antitumor and anti-inflammatory assays. The results revealed that compounds 1, 2, and 3 all showed inhibitory activity toward three tumor cell lines, A549 (human lung carcinoma), BEL-7402 (human liver carcinoma) and HCT-8 (human colon carcinoma). None of the compounds exhibited anti-inflammatory activity.

1. Introduction

Lindera reflexa Hemsl is a type of shrub or vine in the family of Lauraceae, mainly distributed in the southern region of the Yangtze River in China. The root of *L. reflexa* Hemsl, which is used for the treatment of gastritis and peptic ulcers, was a newly discovered herbal drug and was listed in the Dictionary of Chinese Medicine. The root was the main ingredient of a Chinese patent medicine for treating peptic ulcers and has been used in clinical practice for the past few years. Previous phytochemical studies showed that the root contains volatile oil [1]; alkaloids of launobine, lindcarpine and laurolitsine [2,3]; flavonoids of pinostrobin, pinocembrin, sakuranetin; and 3, 5-didroxystilbene [4,5].

To further study this herbal medicine, our lab has conducted a great deal of research on the volatile oil as well as on water, petroleum ether, ethyl acetate and n-butanol extracts. Additionally, pharmacological evaluations of the anti-inflammatory, analgesic, and anti-ulcer activities of these extracts have been performed. Ultimately, the ethyl acetate and n-butanol extracts were found to have significant anti-ulcer and anti-inflammatory activities. In this study, the ethyl acetate and n-butanol extracts were analyzed. At the same time, 14 compounds were obtained, among which compounds 1, 2 and 3 were newly discovered. Compound 1, named reflexan A, was found to possess a novel skeleton. Compound 2 and compound 3, named reflexanbene I and reflexanbene II, respectively, were newly discovered compounds fused to stilbene, and the structure of the new compounds is shown in Fig. 1. Their antitumor activities and anti-inflammatory potencies have been investigated in various systems to date.

2. Results and discussion

Reflexan A (compound 1) was obtained as colorless needles. mp.164–167 °C; $[\alpha]_D^{20} = -2.2°$ (c 0.09, $CHCl_3$);and was soluble in chloroform and slightly soluble in methanol. Upon addition of the test solution of ferric chloride–potassium ferricyanide, the compound turned blue, which suggested that the compound containing phenol groups. The molecular formula was determined to be $C_{27}H_{34}O_5$ by HR ESI-MS at m/z: 439.2490$[M + H]^+$ (calcd 439.2484), which indicated the compound contained 11 degrees of unsaturation. The UV spectrum showed the maximum absorption wavelength at 298.4 nm. IR absorptions indicated the existence of a hydroxyl group (3433 cm^{-1}), ketone (1713 cm^{-1}, 922 cm^{-1}), olefin (1660 cm^{-1}), and an aromatic ring (1575 cm^{-1}, 1496 cm^{-1}, 1455 cm^{-1}).

The ^{1}H-NMR spectrum (Table 1) displayed five protons signals at δ7.13–7.27, δ7.15 (3H,t,J = 7.2,7.0Hz) and 7.24 (2H,m), which are typical mono-substituted benzene ring proton signals. The signals at δ5.94 (1H,s) and δ4.98 (1H,s) were proton signals of a double bond, and each of the two carbons were substituted; the signals at δ1.55 (3H,s), 1.93 (3H,s), 0.69 (3H,d), and 0.79 (3H,d) corresponded to four methyl groups.

The ^{13}C-NMR spectrum (Table 1) displayed fourteen carbon signals, including a benzene ring, two carboxyls, and two double bond carbons. The remaining two carbon signals were indicative of sp2-hybridized quaternary carbons (C-3,C-4). Thirteen carbon signals were observed downfield, among which δ70.6 was located upfield and suggested a carbon bonded to an oxygen atom. Together with the DEPT135 spectrum,

* Corresponding author at: Henan University of Traditional Chinese Medicine, BoXue Road, Jinshui District, ZhengZhou 450046, China.
E-mail addresses: suiqingchen@163.com (S. Chen), wllywlly2004@163.com (L. Wang).

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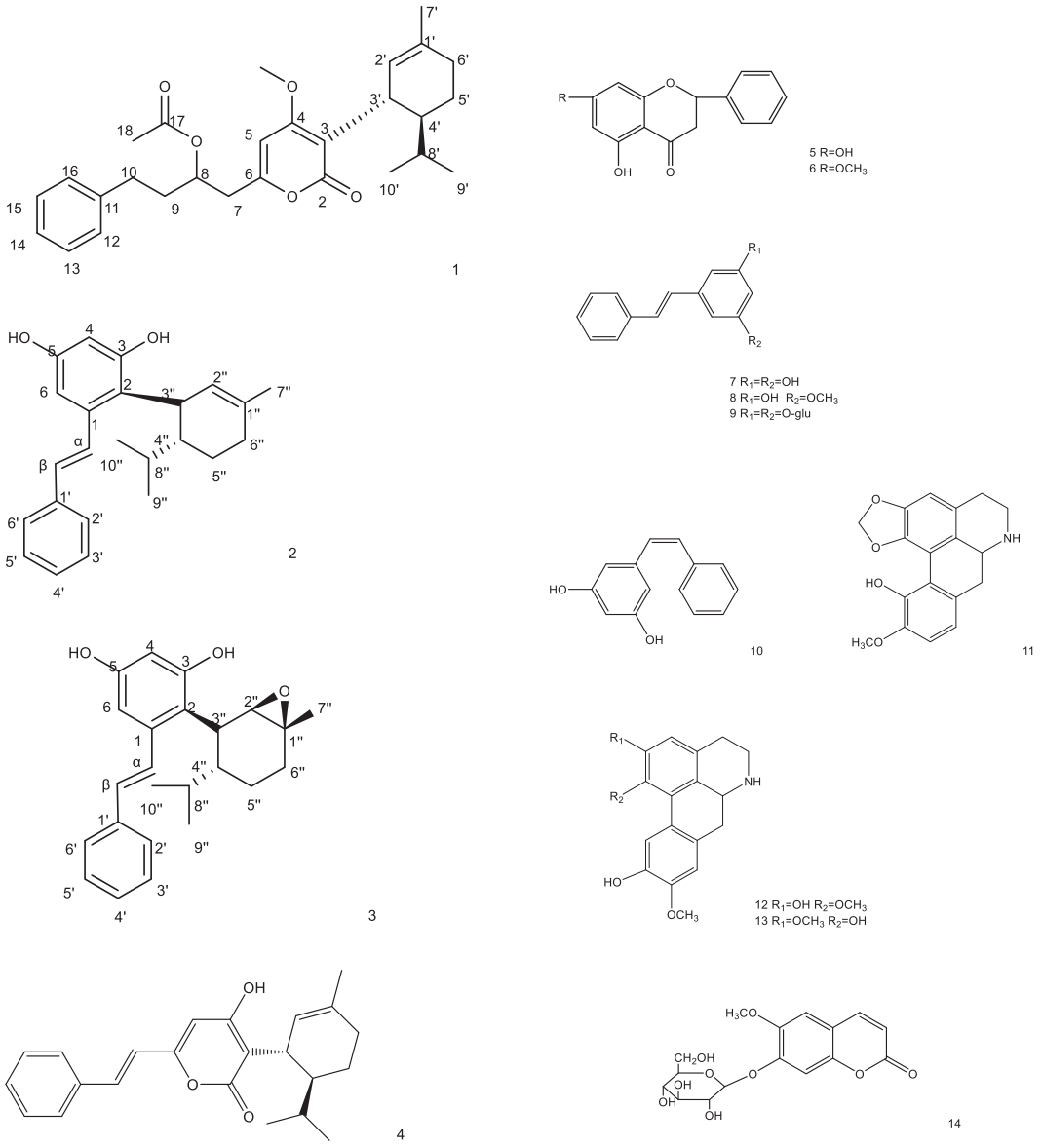

Fig. 1. New compounds from *Lindera reflexa* Hemsl.

the results indicated the presence of five methylenes, four methines and four methyls.

The HMBC spectrum (Table 2) showed correlations of δ0.69 (H-10′) and 0.79 (H-9′), with δ28.4 (C-8′), and 39.9 (C-4′) and δ1.55 (H-7′), with δ132.2 (C-1′), 125.2 (C-2′), and 30.5 (C-6′), and indicated that compound 1 might be a C-3-substituted menthene derivative, which was also confirmed by the ^{1}H–^{1}H COSY spectrum.

δ5.94 (H-5) correlated to δ104.2 (C-3) and 159.4 (C-6), and δ3.37 (H-3′) exhibited long-range correlations with δ104.2 (C-3), 163.5 (C-4) and 165.3 (C-2). These correlations, combined with the mono-substituted benzene ring proton signals in the ^{1}H NMR spectrum, suggested that a pyran-2-one group existed in the compound. These data were similar to those of the pyran-2-one moiety of katsumadain [6], and C-3′ linked to C-3.

The ^{1}H-^{1}H COSY spectrum showed the correlations of H-7 with H-8, H-8 with H-9, and H-9 with H-10, and the HMBC spectrum showed the correlations of δ4.96 (H-8) with δ169.9 (C-17), and δ2.60 (H-10) with δ70.6 (C-8), which indicated the existence of a —$CH_2CH_2CH_2OCOCH_3$ moiety [7]. In contrast to katsumadain, compound 1 had no alkenyl group at C-7 and contained a —$CH_2CH_2CH_2OCOCH_3$ moiety.

δ2.60 (H-10) exhibited long-range correlations with δ141.3 (C-11), 128.4 (C-12, 16), 70.6 (C-8) and 35.1 (C-9), indicating a benzene ring linked to C-10, δ2.65 (H-7), which had long-range correlation with 159.4 (C-6) and indicated C-7 was connected to C-6 of the pyran-2-one moiety.

The NOE spectrum (Fig. 2) showed that C-3′ and C-4′ represented a trans-oriented structure because H-3′ and H-4′ had no NOE correlations.

The absolute configuration was determined by comparing the experimental and calculated CD spectra. The latter was performed using time-dependent density functional theory. Optimized geometries were obtained by systematic conformational analysis using the MMFF94 force field, and ECD spectra were calculated in methanol solution using the B3LYP/6–31 g (d) level of theory with the CPCM model. The results showed that the experimental and calculated spectra were in good agreement (Fig. 3). Thus, the absolute configuration of 1 was determined to be 3′S,4′S.

In summary, the compound was determined to be 4-hydroxy-3-[(3′R,4′R)-1′-p-mentheneyl]-6-(4-phenyl-2-acetoxy group-n-butyl)-2H-pyran-2-one.