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Anti-inflammatory lignans and phenylethanoid glycosides from the root of *Isodon ternifolius* (D.Don) Kudô

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ABSTRACT

Five undescribed lignans, three undescribed phenylethanoid glycosides and eight known compounds were isolated from the root of *Isodon ternifolius* (D.Don) Kudô (Lamiaceae). The structures of all of the isolated constituents were characterized by physical data analyses including NMR, MS and ECD. The anti-inflammatory activities of the isolates were evaluated based on their ability to inhibit NO, PGE2 and TNF-α production in LPS-induced RAW 264.7 macrophage cells. Six phenyl-naphthalene lignans, ternifoliuslignan A, ternifoliuslignan B, ternifoliuslignan C, ternifoliuslignan D, ternifoliuslignan E and 3-carboxy-6,7-dihydroxy-1-(3′,4′-dihydroxyphenyl) -naphthalene, can substantially inhibit the release of NO with IC_{50} values in the range of 9.98–29.14 μM, which are better than the positive reference. These phenyl-naphthalene lignans could markedly decrease the secretions of PGE2 and TNF-α in LPS-induced RAW264.7 cells. Ternifoliuslignan C and ternifoliuslignan D decreased iNOS, COX-2 and NF-κB/p65 protein expression. A preliminary structure-activity relationship among the phenyl-naphthalene lignans for the anti-inflammatory activity was discussed.

1. Introduction

The genus *Isodon* consists of approximately 150 species that are mainly distributed in the tropical and subtropical regions of Asia (Delectis Florae reipublicae popularis sinicae agendae academiae sinicae edita). The genus is famous for having been the source of more than 1200 new diterpenoids with diverse skeletons (Liu et al., 2017; Zou et al., 2012). Some species have been used in traditional medicine, e.g., *Isodon japonica* (Burm.f.) H. Hara has been traditionally used to treat hepatitis, gastritis, mastitis, stomach-aches and arthralgia (Chi et al., 2016); *Isodon rubescens* (Hemsl.) H. Hara has been used in traditional Chinese medicine (TCM) for its various activities, including anti-inflammatory, antitumour, antimicrobial, immunological and antioxidant activities, as well as its hypotensive effects (Guo et al., 2010); and the roots and aerial parts of *Isodon ternifolius* (D.Don) Kudô have been used in TCM to treat diarrhoea, enteritis, acute icterohepatitis, and other types of inflammation (Wu and Li, 1977). *I. ternifolius* is also a major ingredient of a Chinese patent medicine “Fufang Sanye xiangchacai Pian”, which is used to treat chronic and acute hepatitis and hepatitis B (Liu et al., 2017).

The anti-inflammatory potential of *Isodon* has been previously reported (Shin et al., 2004a, b; Hong et al., 2007; Lee et al., 2007). In our first study, the ethyl acetate extract of *I. ternifolius* showed decreased secretions of NO in LPS-induced RAW264.7 cells. To inspire further use of this plant and identify new anti-inflammatory agents, further investigations were carried out. The structures of all isolated constituents were characterized by comprehensive spectroscopic analyses, and the absolute configurations were elucidated by quantum chemical CD calculations. These findings have led to the isolation of five undescribed lignans (**1–5**), three undescribed phenylethanoid glycosides (**6–8**), three known lignans (**9**−**11**), three phenylpropanoids (**12–14**) and two phenylethanol derivatives (**15–16**) (Fig. 1). The anti-inflammatory effects of all isolates were evaluated with regard to their activities on the production of NO and pro-inflammatory cytokines TNF-α and PGE2 in vitro by using LPS-activated RAW264.7 macrophage cells. Furthermore, the anti-inflammatory mechanisms of some compounds were clarified by western blot analysis. We also explored the structure−activity relationships among some lignans obtained from *I. ternifolius*. The

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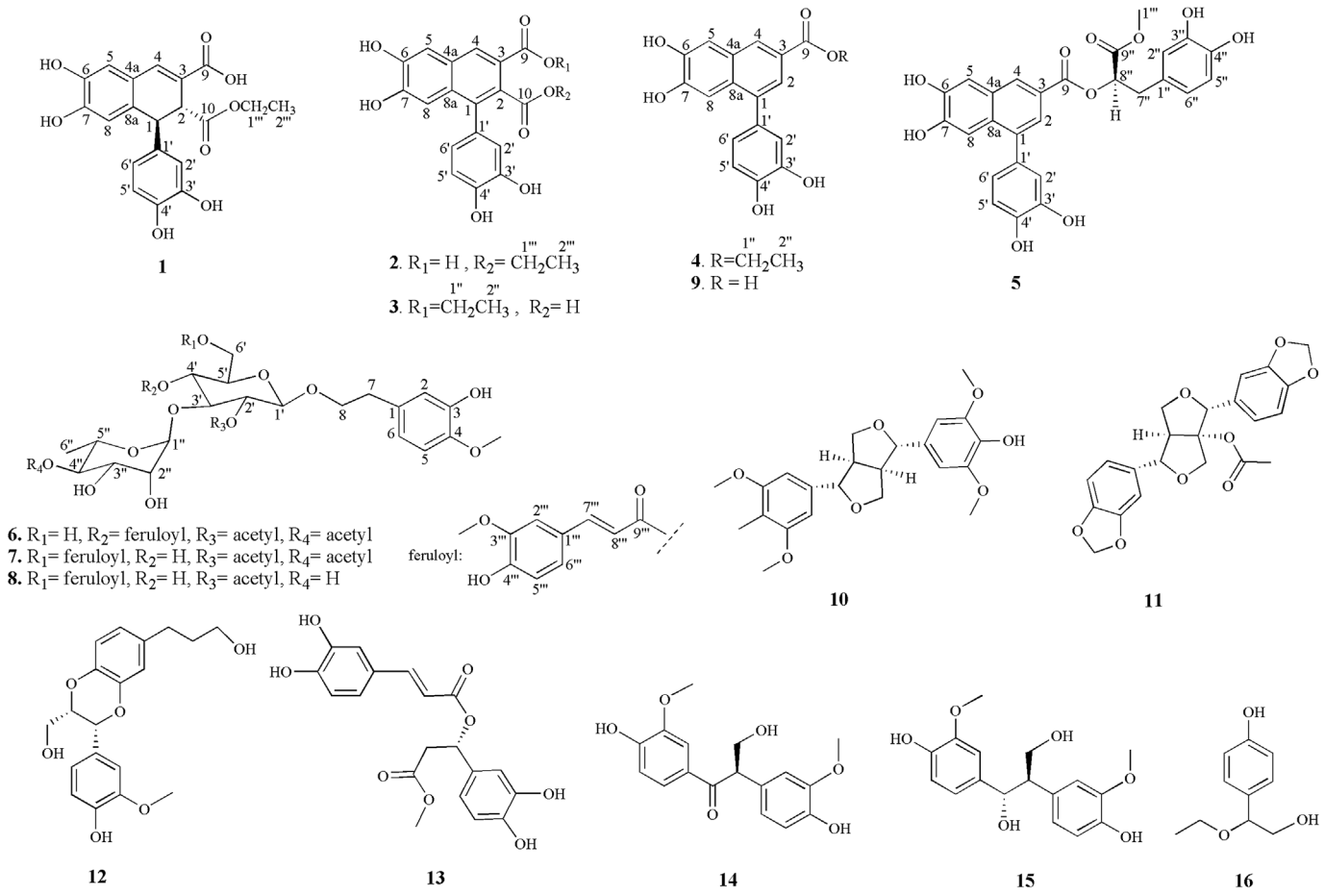

Fig. 1. Structures of compounds **1–16**.

research provided the basis for expanding the utilization and development of this medicinal plant.

2. Results and discussion

2.1. Structural elucidation of the new compounds

The molecular formula of ternifoliuslignan A (**1**) was determined as $C_{20}H_{18}O_8$ based on the sodium adduct ion at *m/z* 409.0896 $[M + Na]^+$ (calcd for $C_{20}H_{18}O_8Na$ 409.0899) in its HRESIMS, which suggests 12 degrees of unsaturation. The ^1H NMR spectrum showed eight methine protons [δ_H 4.34 (d, J = 4.3 Hz), 3.84 (d, J = 4.3 Hz); 6.47 (d, J = 2.1 Hz), 6.65 (d, J = 8.1 Hz) and 6.40 (dd, J = 8.1, 2.1 Hz) attributed to a 1,2,4-trisubstituted aromatic moiety and three singlets at δ_H 7.57, 6.82, and 6.50 (1H each)]; one oxygenated methylene proton at δ_H 4.04 (q, J = 7.1 Hz); and one methyl signal at δ_H 1.12 (t, J = 7.1 Hz). The coupling constant of the methyl signal (7.1 Hz) indicated the presence of an ethoxy group (Table 1). The ^{13}C NMR spectrum showed 20 signals comprising two carboxylic carbons (δ_C 170.7 and 175.0), fourteen aromatic or olefinic carbons, and four sp^3 carbons (two methine carbons, one methylene carbon and one methyl carbon attributed to an ethoxy group) (Table 2). These data are similar to those of a phenyldihydronaphthalene moiety (Chawla et al., 1992). In the HMBC experiment, the correlations from H-1 to C-2′, C-6′, C-8 and C-10; H-4 to C-2, C-5, C-8a and C-9; and H-1‴ to C-10 showed that the ethoxy group was linked to C-10 (Fig. 2). The relative configuration of **1** was inferred from coupling constant between H-1 and H-2 and a ROESY experiment. The coupling constant (J = 4.3 Hz) between H-1 and H-2 was in accordance with a relative 1,2-trans-configuration (Ma et al., 2007), moreover, the ROESY spectrum of compound **1** showed correlation between the signals at δ_H 4.34 (H-1) and δ_H 6.50 (H-8) as well as between the signals at δ_H 3.84 (H-2) and δ_H 6.47 (H-2′) (Fig. 3) which suggested a trans relative configuration between H-1 and H-2 (Fig. 3) (Ren et al., 2017). Furthermore, the absolute structure of **1** was confirmed by the ECD spectrum, which showed a positive Cotton effect at 209 and 373 nm and a negative Cotton effect at 240 and 343 nm. The experimental ECD spectrum of **1** coincided with the calculated ECD spectrum of (1*S*,2*R*)-**1** (Fig. 4), which are similar to those of compound **2b** [dimethyl (1*S*,2*R*)-1-(3,4-dimethoxyphenyl)-6,7-dimethoxy-1,2-dihydronaphthalene-2,3-dicarboxylate] (Nishizawa et al., 1990) and compound **4a** [dimethyl (1*S*,2*R*)-1-(3,4-dihydroxyphenyl)-6,7-dihydroxy −1,2-dihydronaphthalene-2,3-dicarboxylate] (Ren et al., 2017). Thus, compound **1** was identified as (1*S*,2*R*)-2,3-dicarboxy-6,7-dihydroxy-1-(3′,4′-dihydroxy)-phenyl-1,2-dihydro-naphthalene-10-ethyl ester and named ternifoliuslignan A.

Ternifoliuslignan B (**2**) was determined to have the formula $C_{20}H_{16}O_8$ based on the sodium adduct ion at *m/z* 383.0754 $[M - H]^-$ (calcd for $C_{20}H_{15}O_8$ 383.0767) in its HRESIMS, which indicates 13 degrees of unsaturation. Compared to compound **1**, compound **2** is missing two protons and has one additional degree of unsaturation, which suggested the presence of a C-1/C-2 double bond. This feature was confirmed by the NMR spectra (Table 2). The ^{13}C NMR signal of C-1 shifted from δ_C 47.3 in **1** to δ_C 137.8 in **2**, and C-2 shifted from δ_C 49.4 in **1** to δ_C 129.8 in **2**. In the HMBC spectrum, the correlation from H-1‴ to C-10 showed that the ethoxy group was linked to C-10. Meanwhile, the correlations from H-6′ to C-1, C-2′, and C-4′ as well as the correlations from H-2′ to C-1, C-4′ and C-6′ showed that the 3′,4′-dihydroxyphenyl moiety was linked to the naphthalene (Fig. 5). Thus, the structure of **2** was established to be 2,3-dicarboxy-6,7-dihydroxy-1-(3′,4′-dihydroxy)-phenylnaphthalene-10-ethyl ester and named