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A new anti-inflammatory lignan from *Lonicerae Japonicae* flos

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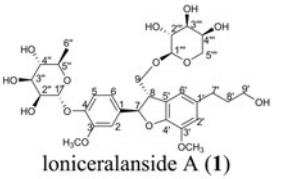
ABSTRACT

One new lignan, loniceralanside A (**1**), together with three known compounds (**2–4**), were purified from *Lonicerae Japonicae* Flos. Their structures were determined by HR-ESI-MS, 1 D and 2 D NMR data. Compounds **2–4** were separated from the plant for the first time. The biological test showed that compound **1** had significant anti-inflammatory activity with inhibiting the release of β-glucuronidase induced by platelet-activating factor (PAF) in rat polymorphonuclear leukocytes (PMNs) ($IC_{50} = 3.05\,\mu M$).

Lonicera Japonica Thunb.



loniceralanside A (**1**)

significant anti-inflammatory activity($IC_{50} = 3.05\,\mu M$)

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Lonicerae Japonicae Flos; lignan; anti-inflammatory activity; β-glucuronidase

1. Introduction

The genus *Lonicera* Linn. consists of more than 200 species and is widely distributed in temperate and subtropical regions (Sun 2012). *Lonicerae Japonicae* Flos is a very common species with highly economic and medicinal values. It has been widely used in the pharmaceutical, cosmetic and functional food industries with varieties of biological properties, such as antioxidant, anti-inflammatory, anti-virus, antitumor and antihypertensive activities (Han et al. 2016; Yu et al. 2016; Chaowuttikul et al. 2017; Ding et al. 2017; Wang et al. 2017). To date, more than 210 compounds have been isolated from *L. Japonicae* Flos including flavonoids, triterpenoids, iridoids, volatile oils and organic acids (Wang et al. 2014; Wang et al. 2016). As part of our ongoing project to study the bioactive constituents in *L. Japonicae* Flos, in the present

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study, one new lignan, loniceralanside A (**1**), together with three known compounds, forsythialanside C (**2**), (7*S*, 8*S*)-3-methoxy-3′,7-epoxy-8,4′-oxyneoligna-4,9,9′-triol (**3**) and eugenyl-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**4**), were isolated from *L. Japonicae* Flos. The bioassays showed that compounds **1–4** expressed anti-inflammatory activities with IC_{50} values ranging from 3.05 to 27.80 μM, and the activity of **1** was comparable to the positive control. In addition, compounds **2–4** were firstly separated from *L. Japonicae* Flos. Herein, we report the isolation, structural elucidation and biological assays of these compounds.

2. Results and discussion

Compound **1** was obtained as a yellow colloid. Its molecular formula was assigned as $C_{31}H_{42}O_{14}$. The IR spectral data indicated the presence of hydroxyl (3428 cm^{-1}) and aromatic ring (1624, 1506 and 1459 cm^{-1}) groups. Its ^1H NMR spectrum showed signals for a 1, 3, 4, 5-tetrasubstituted aromatic ring with two phenyl protons [δ_H 6.76 (1H, br s, H-2′), 6.76 (1H, br s, H-6′)], and a 1, 3, 4-trisubstituted aromatic ring with three phenyl protons [δ_H 7.08 (1H, s, H-2), 7.09 (1H, d, $J = 8.3$ Hz, H-5), 6.96 (1H, dd, $J = 1.8$, 8.3 Hz, H-6)]. Signals for two methoxyl groups [δ_H 3.89 (3H, s, OCH_3-3′), 3.83 (3H, s, OCH_3-3)], two oxymethylene groups [δ_H 3.88 (1H, m, H-9_a), 3.76 (1H, m, H-9_b), 3.89 (1H, m, H-$9'_a$), 3.57 (1H, m, H-$9'_b$)], two methylene groups [δ_H 2.64 (2H, m, H-7′), 1.82 (2H, m, H-8′)], a oxymethine group [δ_H 5.67 (1H, d, $J = 5.8$ Hz, H-7)], and a methine group [δ_H 3.63 (1H, m, H-8)] were also observed. The ^{13}C NMR spectrum exhibited the presence of six signals for a rhamnosyl group and five signals for an arabinosyl group. The key ^1H-^1H COSY correlations showed the presence of CH(7)-CH(8)-CH_2(9) and CH_2(7′)-CH_2(8′)-CH_2(9′) groups (Supplementary Material Figure S1). In the HMBC spectrum, the correlations from H-7 to C-2 and C-6, and from H-8 to C-1 indicated that the 1, 3, 4-trisubstituted aromatic ring was located at C-7. The correlations from H-7′ to C-1′, C-6 ′ and C-2′ confirmed that the propanol group was linked to C-1′. The HMBC correlations from H-7 to C-4′, and from H-8 to C-4′ and C-6′ indicated the presence of a dihydrobenzofuran group. In addition, the rhamnosyl and arabinosyl groups were located at C-4 and C-9 respectively, according to the HMBC correlations from H-1″ to C-4 and from H-1‴ to C-9 (Supplementary Material Figure S1).

The relative configuration of C-7 and C-8 was determined as a relative-trans form by the chemical shift and coupling constant value of H-7 [δ_H 5.67 (1H, d, $J = 5.8$ Hz, H-7)], and the NOESY correlations of H-7/H-9, H-8/H-2 and H-8/H-6 (Supplementary Material Figure S2). Then, in the experimental ECD data, **1** showed a negative cotton effect at 282 nm (-1.02) (Supplementary Material Figure S11), so the configuration of the dihydrofuran ring was determined as 7*R*, 8*S* (Antus et al. 2001; Huang et al. 2015). The relative configuration of glycosyl groups in **1** were determined to be α for both rhamnosyl and arabinosyl units on the basis of chemical shifts and coupling constant values [δ_H 5.35 (1H, d, $J = 1.6$ Hz, H-1″), 4.34 (1H, d, $J = 7.3$ Hz, H-1‴)] (Wang et al. 2016; Yan et al. 2017). Acid hydrolysis of **1** yielded L-rhamnose and L-arabinose as sugar units (Supplementary Material Figure S12). Thus, the structure of **1** was determined to be (7*R*, 8*S*)-dihydrodehydrodiconiferyl alcohol-4-O-α-L-rhamnopyranosyl-9-O-α-L-arabinopyranoside, and named as loniceralanside A (Figure 1).

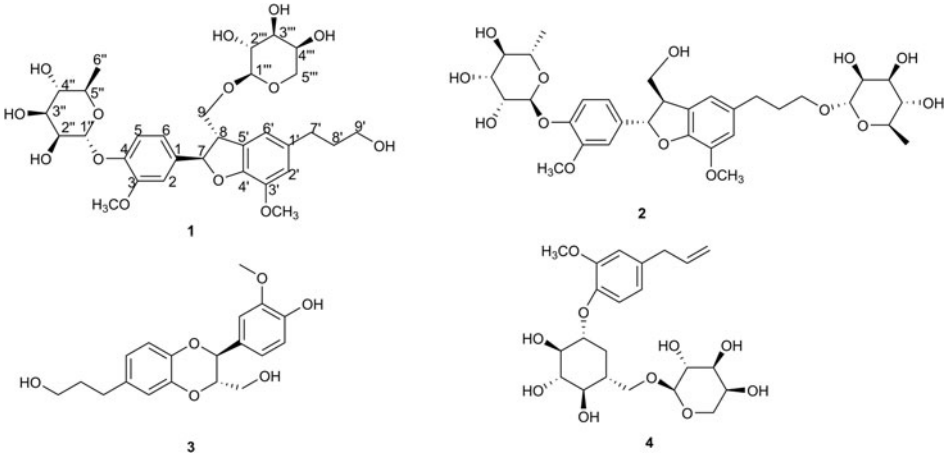

Figure 1. Chemical structures of compounds **1–4**.

The three known compounds were identified as forsythialanside C (**2**) (Li et al. 2014), (7*S*, 8*S*)-3-methoxy-3′,7-epoxy-8,4′-oxyneoligna-4,9,9′-triol (**3**) (Chang et al. 2014) and eugenyl-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**4**) (Nakamura et al. 2011) by comparing their spectroscopic data with the literature.

As far as we know, lignans have varieties of structures such as benzofuran, bicyclooctene, futoenone and biphenyl derivative lignans. In addition, they display a wide range of significant biological properties, such as anti-inflammatory, antitumor, antimicrobial and antioxidant activities (Geethangili and Ding 2018; Xu et al. 2018). In the present study, the anti-inflammatory activities of compounds **1**–**4** were evaluated (Supplementary Material Table S2). Compounds **1** and **2** were benzofuran lignan glycosides, and showed comparable activities (**1**: $IC_{50} = 3.05\,\mu M$; **2**: $IC_{50} = 4.31\,\mu M$) to that of the positive control ($IC_{50} = 2.21\,\mu M$). Their activities appeared to be related with the structures. Comparing the structures and activities of **1** and **2** with those of the reported lignan hawthornnin H which possessed two hydroxyl groups at C-7′ and 8′ (Huang et al. 2015), it seemed that the two methylene groups of C-7′ and 8′ in **1** and **2** might lead to better anti-inflammatory activities. Comparing **1** and **2** with the known benzofuran lignan difengpiol B which possessed a partially hydrogenated aromatic ring (Fang et al. 2010), it seemed that the 1, 3, 4-trisubstituted aromatic rings and glycosyl substituents in **1** and **2** might be important for higher activities. Moreover, compound **3** was an oxyneolignan and **4** was a phenylpropanoid glycoside, both with mild anti-inflammatory activities (**3**: $IC_{50} = 15.30\,\mu M$; **4**: $IC_{50} = 27.80\,\mu M$).

3. Experimental

3.1. General experimental procedures

Optical rotations were obtained on a Rudolph VI automatic polarimeter (Rudolph Research Analytical, Hackettstown, USA). IR spectra were recorded on an FT-IR VERTEX 70 spectrometer (Bruker BioSpin AG, Fremont, USA). NMR experiments were performed with a Bruker AVANCE DRX (600 MHz) spectrometer (Bruker BioSpin Corporation, Billerica, USA). ESIMS data were measured on an Agilent 1260-6460 Triple Quad LC-MS

spectrometer (Agilent Technologies, Santa Clara, USA). HRESIMS data were measured on an Agilent 6545 Q-TOF mass spectrometer (Agilent Technologies, Santa Clara, USA). HPLC preparation was carried out on an Agilent 1260 instrument (Agilent Technologies, Santa Clara, USA). Sugar analysis was carried out using a Shodex Asahipak NH_2P-50 column (5 μm, 4.6 $\times$ 250 mm; Shodex Denko, Tokyo, Japan). All solvents used for column chromatography were obtained with analytical grade (Fuyu Chemical Factory, Tianjin, China).

3.2. Plant material

The initial flowers of *L. Japonicae* were obtained from Shandong University of Traditional Chinese Medicine (Jinan, China), and were identified by Prof. Jia Li (Shandong University of Traditional Chinese Medicine, Jinan, China). A voucher specimen (JYH-201705) was stored in the Laboratory of Pharmaceutical Chemistry, University of Jinan (Jinan, China).

3.3. Extraction and isolation

The freeze-dried flowers (6.0 kg) were extracted with 95% ethanol (30.0 L) to obtain crude extract (900.5 g). The extract was then extracted with ethyl acetate (10.0 L) and *n*-butanol (12.0 L). The *n*-butanol fraction (65.2 g) was chromatographed over a macroporous resin column (EtOH-H_2O, 0:100 to 90:10, *v/v*) to yield six fractions (A-F). Fraction B (10.4 g) was chromatographed over a silica gel column (CH_2Cl_2-MeOH, 10:1 to 1:1, *v/v*) to afford fractions B1-B7. Fr.B4 (1.1 g) was subjected to silica gel column chromatography eluted with EtOAc-MeOH (130:1 to 30:1, *v/v)* to obtain night subfractions Fr.B4-1–B4-9. Fr.B4-3 (38.5 mg) was purified through preparative HPLC (MeOH-H_2O, 40:60, *v/v*), yielding compounds **1** (3.4 mg), **2** (2.5 mg) and **4** (12.1 mg). Fr.B7 (2.0 g) was separated by a silica gel column eluted with EtOAc-MeOH (150:1 to 30:1, *v/v*) to obtain five subfractions Fr.B7-1–B7-5. Fr.B7-4 (41.5 mg) was purified by preparative HPLC eluted with MeOH-H_2O (50:50, *v/v*) to afford compound **3** (17.0 mg).

3.3.1 Compound 1

Yellow colloid; $[\alpha]_D^{25}$ -60.1 (*c* 0.7, CH_3OH); IR (KBr) ν_{max}: 3428, 2927, 1624, 1506, 1459, 1066 cm^{-1}; ^1H NMR (methanol-d_4, 600 MHz) data, see Supplementary Material Table S1; ^{13}C NMR (methanol-d_4, 150 MHz) data, see Supplementary Material Table S1; ESIMS *m/z* 661.2 $[M + Na]^+$; HRESIMS *m/z* 673.2266 $[M + Cl]^-$ (calcd for $C_{31}H_{42}ClO_{14}$, 673.2263).

3.4. Acid hydrolysis of compound 1

The absolute configurations of glycosyl units in compound **1** were determined by the reported method (Yan et al. 2017). Briefly, the sample solution of **1** was firstly hydrolyzed by hydrochloric acid at 80 °C for 4 h. Then the reaction system was neutralized and extracted with CH_2Cl_2. The aqueous layer was analyzed by HPLC with acetonitrile and water (75: 25) at a flow of 1.0 mL/min. Compound **1** gave L-rhamnose

($t_R = 13.30$ min) and L-arabinose ($t_R = 6.98$ min) as sugar residues on the basis of the same retention times with those of authentic L-rhamnose ($t_R = 13.30$ min) and L-arabinose ($t_R = 6.98$ min).

3.5. Anti-inflammatory activity assay

The anti-inflammatory activities were assayed by the method that we described previously (Yang et al. 2019).

4. Conclusions

In conclusion, one new lignan, loniceralanside A (**1**), along with three known compounds (**2**-**4**), were separated from *L. Japonicae* Flos. Compounds **2-4** were purified from the plant for the first time. Compound **1** expressed decent anti-inflammatory activity with an IC_{50} of 3.05 μM. This work provided valuable support for the further study of *L. Japonicae* Flos.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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