Fitoterapia 140 (2020) 104417

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Inhibition of IL-6 expression by lignans and other constituents isolated from *Schefflera rubriflora* C. J. Tseng & G. Hoo

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ARTICLE INFO

Keywords:
Schefflera rubriflora
Lignans
Pyran derivatives, IL-6 inhibition

ABSTRACT

Schefflera rubriflora, a plant native to Yunnan Province in China, is often used to treat ailments such as neuropathic pain, tracheitis, and cough. However, the active components imparting these pharmacological effects are largely unexplored. In this study, five novel lignans and three new derivatives of benzoid or pyran were isolated from the leaves and twigs of *S. rubriflora*. The structures of these compounds were determined by the comprehensive analyses of the 1D and 2D NMR spectra and ESI mass spectra and a comparison of the obtained data with those of the literature data. All the compounds were tested for the inhibition of IL-6 expression. Three of the isolated compounds could inhibit the expression by 52% to 72%.

1. Introduction

Schefflera rubriflora C. J. Tseng & G. Hoo is a plant distributed in Xishuangbanna in the Yunnan Province of China [1]. It is traditionally used to treat neuropathic pain, tracheitis, cough, and rheumarthritis [2]. However, the knowledge of the active compounds present in this plant and their pharmacological activities are limited. Previously, we reported that the EtOAc and *n*-BuOH fractions of the 95% ethanol extract of *S. rubriflora* showed 35.7% and 40.6% inhibitions of the croton oil-induced ear inflammation in mice, from which eight new terpenes isolated exhibited tumor necrosis factor-*α* (TNF-*α*) and interleukin-6 (IL-6) inhibitory activities [3].

The leaves and twigs of *Schefflera kwangsiensis*, the other species in the genus *Schefflera*, are conventionally made into the Campo Peach Twig Tablets and widely used in China to treat inflammation and pain [4]. The diverse pharmacological activities of the species in this genus inspired us to further study the phytochemistry and bioactivity of *S. rubriflora*. In the present study, five novel lignans (**1**–**5**) and three derivatives of benzoid (**6**) or pyran (**7**,**8**) were isolated from the *n*-BuOH fraction of *S. rubriflora*. Herein, details of the isolation, structural elucidation, and bioactivities of these compounds are described.

2. Materials and methods

2.1. Instrumentation

Optical rotation was measured using a Jasco P-2000 digital polarimeter (Jasco, Tokyo, Japan). UV spectra were collected in methanol on a Jasco V-650 UV–Vis spectrophotometer. Fourier-transform infrared (FTIR) spectroscopy was performed on a Nicolet 5700 ATR-FTIR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). ^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance III 400 MHz (or 500 MHz) NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) or an Agilent VNMRS 600 MHz NMR spectrometer (Palo Alto, CA, USA). HRESIMS were recorded on an Agilent 1200 SL series LC/6520 QTOF spectrometer (Agilent, Boblingen, Germany) or a Thermo Fisher Scientific Q Exactive Focus Hybrid Quadrupole-Orbitrap mass spectrometer (Waltham, MA, USA). Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Samples were fractionated on C-18 columns (50 μm; YMC, Kyoto, Japan). Fractions were analyzed by HPLC (Agilent, Boblingen, Germany).

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https://doi.org/10.1016/j.fitote.2019.104417
Received 26 September 2019; Received in revised form 30 October 2019; Accepted 4 November 2019
Available online 07 November 2019
0367-326X/

2.2. Plant material

Leaves and twigs of *Schefflera rubriflora* C. J. Tseng & G. Hoo (Araliaceae) were collected from Xishuangbanna District (GPS coordinates: N 21°56′-22°17′, E 100°51′-101°04′), Yunnan Province, China, in June 2013 (summer, wet season) and were identified by Professor Lin Ma of the Institute of Materia Medica, Chinese Academy of Medical Science and Peking Union Medical College, China. A voucher specimen (ID-S-2478) was deposited in the Institute of Materia Medica, Chinese Academy of Medical Science and Peking Union Medical College, China.

2.3. Extraction and isolation

Air-dried, powdered leaves and twigs from *S. rubriflora* (20.1 kg) were extracted with 95% EtOH (3 × 50 L) under refluxing conditions for 1.5 h. The EtOH extract was evaporated under reduced pressure to afford the crude extract (1.5 kg), which was then suspended in H_2O and successively partitioned with petroleum ether, EtOAc, and *n*-BuOH.

The *n*-BuOH fraction (510 g) was subjected to chromatography on a Diaion HP-20 macroporous resin column and eluted with H_2O, followed by 10%, 30%, 50%, 70%, and 95% EtOH. The fraction corresponding to 50% EtOH (20 g) was further loaded onto a C-18 column and eluted with a MeOH–H_2O (10:90 → 100:0) gradient to yield nine fractions (1–9). Separation of fraction 3 (1.5 g) with Sephadex LH-20 column (2 × 120 cm, MeOH–H_2O, 3:7) and preparative HPLC (Agilent ZORBAX Eclipse XDB-C18, 5 μm, 9.4 × 250 mm), successively yielded compounds **7** (10 mg, MeOH–H_2O, t_R = 33 min), **5** (27 mg, MeOH–H_2O, t_R = 42 min), and **8** (12 mg, MeOH–H_2O, t_R = 50 min). Fraction 5 (2.0 g) was further loaded onto a Sephadex LH-20 column (2 × 120 cm, MeOH–H_2O, 3:7) and purified by semi-preparative HPLC (Agilent ZORBAX Eclipse XDB-C18, 5 μm, 9.4 × 250 mm), eluted with MeOH–H_2O (4:6) to yield compounds **4** (12 mg, t_R = 23 min), **3** (15 mg, t_R = 33 min), **6** (7 mg, t_R = 35 min), **2** (60 mg, t_R = 40 min), and **1** (18 mg, t_R = 43 min).

2.3.1. Characterization of (7S,8R)-7′,8′-threo-4-O-1″,3″-dihydroxy-2″-propyl bennettin (1)

White powder; $[\alpha]_D^{20}$ - 35.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 208 nm (1.98), 238 nm (1.01); IR ν_{max}: 3315, 1597, 1503, 1461 cm^{-1}. ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz): Tables 1 and 2. HRESIMS (positive ion) *m/z* 519.1843 $[M + Na]^+$ (calcd for $C_{24}H_{32}NaO_{11}$, 519.1837). ECD (*c*, 3.36 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$): 207 nm (−2.37), 227 nm (−0.67), 245 nm (+1.59), 286 nm (+0.46).

2.3.2. Characterization of (7S,8R)-7′,8′-threo-meliasendanin D 7′-O-β-D-glucopyranoside (2)

White powder; $[\alpha]_D^{20}$ +36.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 206 nm (1.65), 233 nm (1.04); IR ν_{max}: 3340, 1604, 1494, 1336 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz): Tables 1 and 2. HRESIMS (positive ion) *m/z* 577.1879 $[M + Na]^+$ (calcd for $C_{26}H_{34}NaO_{13}$, 577.1892). ECD (*c*, 1.83 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$): 212 nm (+4.01), 243 nm (+2.68), 286 nm (+0.87).

2.3.3. Characterization of (7R,8S)-9′-O-butyl-5-methoxydehydrodiconiferyl alcohol 4-O-β-D-glucopyranoside (3)

White powder; $[\alpha]_D^{20}$ - 69.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 206 nm (2.02), 274 nm (1.52); IR ν_{max}: 3391, 1598, 1501, 1462 cm^{-1}. ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz): Tables 1 and 2. HRESIMS (positive ion) *m/z* 629.2562 $[M + Na]^+$ (calcd for $C_{31}H_{42}NaO_{12}$, 629.2568). ECD (*c*, 2.72 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$): 218 nm (−0.42), 234 nm (+2.30), 275 nm (−2.04).

2.3.4. Characterization of (7R,8S)-9′-O-butyl-dehydrodiconiferyl alcohol 4-O-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside (4)

White powder; $[\alpha]_D^{20}$ - 92.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 204 nm (1.69), 276 nm (1.27); IR ν_{max}: 3342, 1597, 1513, 1462 cm^{-1}. ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz): Tables 1 and 2. HRESIMS (negative ion) *m/z* 707.2919 $[M - H]^-$ (calcd for $C_{35}H_{47}O_{15}$, 707.2920). ECD (*c*, 4.66 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$): 216 nm (−1.60), 232 nm (+2.61), 282 nm (−4.59).

2.3.5. Characterization of (7S,8R)-9′-O-ethyl-dehydrodiconiferyl alcohol 4-O-β-D-{6″-O-[(1‴S,2‴R,4‴S,6‴R,7‴E,9‴Z)-dihydrophaseic acyl]}-glucopyranoside (5)

White powder; $[\alpha]_D^{20}$ - 89.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 203 nm (1.77), 273 nm (1.48); IR ν_{max}: 3388, 1702, 1602, 1511, 1460 cm^{-1}. ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz): Tables 1 and 2. HRESIMS (positive ion) *m/z* 835.3522 $[M + Na]^+$ (calcd for $C_{43}H_{56}NaO_{15}$, 835.3511). ECD (*c*, 4.06 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$): 232 nm (−5.63), 261 nm (+2.45), 293 nm (+1.58).

2.3.6. Characterization of methyl benzoate 2-O-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside (6)

White powder; $[\alpha]_D^{20}$ - 110.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 203 nm (1.56), 226 nm (1.05); IR ν_{max}: 3345, 1715, 1601, 1491, 1452 cm^{-1}. ^1H NMR (D_2O, 400 MHz) and ^{13}C NMR (D_2O, 100 MHz): Tables 1 and 2. HRESIMS (positive ion) *m/z* 469.1313 $[M + Na]^+$ (calcd for $C_{19}H_{26}NaO_{12}$, 469.1316).

2.3.7. Characterization of 4-(3-hydroxypropyl)-2-oxo-2H-pyran-6-carboxylic acid (7)

White powder; UV (MeOH) λ_{max} (log ε): 205 nm (1.59), 297 nm (1.03); IR ν_{max}: 3397, 1731, 1644, 1562, 1438 cm^{-1}. ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz): Tables 1 and 2. HRESIMS (negative ion) *m/z* 197.0455 $[M - H]^-$ (calcd for $C_9H_9O_5$, 197.0455).

2.3.8. Characterization of butyl 3-(3-hydroxypropyl)-2-oxo-2H-pyran-6-carboxylate (8)

White powder; UV (MeOH) λ_{max} (log ε): 203 nm (2.95), 302 nm (1.05); IR ν_{max}: 3394, 1723, 1643, 1516, 1419 cm^{-1}. ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz): Tables 1 and 2. HRESIMS (positive ion) *m/z* 255.1221 $[M + H]^+$ (calcd for $C_{13}H_{19}O_5$, 255.1227).

2.4. Enzymatic hydrolysis of 2

2.4.1. The hydrolyzation of 2 (2a, meliasendanin D)

Compound **2** (10 mg) was incubated with snailase in sodium acetate buffer (pH 4.5) at 37 °C for 48 h. The mixture was put in a water bath at 90 °C to stop the reaction [5]. Then, it was extracted with *n*-BuOH three times to obtain a fraction containing the aglycone of **2**, which was further separated by preparative HPLC (MeOH–H_2O, 4:6) to give the purified hydrolysate of **2a** (t_R = 49 min, 3.5 mg).

2.4.2. Characterization of hydrolysate of 2 (2a, meliasendanin D)

HRESIMS (positive ion) *m/z* 415.1358 $[M + Na]^+$ (calcd for $C_{20}H_{24}NaO_8$, 415.1363). ^1H NMR (pyridine-d_5, 400 MHz) δ_H (ppm) 7.58 (1H, br s, H-2), 7.44 (1H, br s, H-2′), 7.35–7.26 (3H, overlapped, H-5, 6, 6′), 5.38 (1H, d, *J* = 5.2 Hz, H-7), 4.44 (1H, m, H-7′), 4.26 (2H, m, H-9′), 4.19 (2H, m, H-9), 3.99 (1H, m, H-8′), 3.77 (3H, s, 3′-OCH_3), 3.63 (3H, s, 3-OCH_3), 3.62 (1H, m, H-8). ^{13}C NMR (pyridine-d_5, 100 MHz) δ_C (ppm) 149.2 (C-3), 148.6 (C-4′), 148.5 (C-4), 144.9 (C-3′), 138.3 (C-1′), 134.2 (C-1), 130.3 (C-5′), 120.1 (C-6), 116.9 (C-6′), 116.6 (C-5), 112.8 (C-2′), 111.2 (C-2), 89.0 (C-7), 78.2 (C-8′), 75.3 (C-7′), 64.8 (C-9), 64.7 (C-9′), 56.4 (3-OCH_3), 56.1 (3′-OCH_3), 55.4 (C-8).

Table 1
^{1}H NMR spectroscopic data (400 MHz) of compounds **1–8.**

N_O.	1[a]	2[b]	3[a]	4[a]	5[a]	N_O.	6[c]	7[a]	8[a]
1						1			
2	6.74 s	6.91 s	6.74 s	7.01 d (1.6)	7.02 d (1.2)	2			
3						3	7.26 br d (7.8)	7.18 d (1.6)	
4						4	7.61 td (7.8, 1.2)		7.43 d (6.8)
5		6.87 br s		7.09 d (8.4)	7.07 d (8.4)	5	7.19 td (7.8, 1.2)	6.41 d (1.6)	7.15 d (6.8)
6	6.74 s	6.77 br s	6.74 s	6.91 dd (8.4, 1.6)	6.86 dd (8.4, 1.2)	6	7.78 br d (7.8)		
7	5.61 d (5.6)	5.43 d (5.6)	5.60 d (6.0)	5.58 d (6.0)	5.59 d (5.6)	7			
8	3.49 m	3.43 m	3.48 m	3.50 m	3.47 m	1′	5.27 d (7.2)	2.65 t (2H, 8.0)	2.56 t (2H, 7.6)
9	3.88 m	3.70 m	3.89 m	3.84 m (2H)	3.85 m	2′	3.74 m	1.84 m (2H)	1.81 m (2H)
	3.80 m	3.62 m	3.81 m		3.76 m	3′	3.57 t (8.4)	3.61 t (2H, 6.4)	3.60 t (2H, 6.4)
1′						4′	3.73 m		
2′	6.96 s	6.86 s	6.97 s	6.96 s	6.97 s	5′	3.65 m		
3′						6′	3.94 d (12.6)		
4′							3.78 dd (12.6,5.4)		
5′						1″	5.37 d (3.0)		4.32 t (2H, 6.8)
6′	6.89 s	6.77 s	6.97 s	6.97 s	6.98 s	2″	4.02 d (3.0)		1.72 m (2H)
7′	4.58 d (5.6)	4.55 d (4.8)	6.57 d (16.0)	6.57 d (16.0)	6.58 d (16.0)	3″			1.46 m (2H)
8′	3.68 m	3.68 m	6.20 dt (16.0, 6.0)	6.18 dt (16.0, 6.0)	6.20 dt (16.0, 6.4)	4″	3.89 d (10.0)		0.98 t (3H, 7.2)
9′	3.54 dd (11.6, 4.4)	3.59 m	4.11 d (2H, 6.0)	4.11 d (2H, 6.0)	4.11 d (2H, 6.4)		3.73 d (10.0)		
	3.38 dd (11.6, 6.4)	3.17 m				5″	3.59 s (2H)		
10′			3.49 t (2H, 6.4)	3.49 t (2H, 6.8)	3.57 q (2H, 6.8)	7-OCH_3	3.95 s (3H)		
11′			1.60 m (2H)	1.60 m (2H)	1.22 t (3H, 6.8)				
12′			1.42 m (2H)	1.42 m (2H)					
13′			0.95 t (3H, 7.6)	0.95 t (3H, 7.6)					
1″	3.74 dd (2H, 4.8, 1.0)	4.41 d (6.4)	4.89 d (7.2)	4.97 d (7.6)	4.81 d (7.8)				
2″	4.00 t (4.8)	3.06 m	3.49 m	3.72 t (8.4)	3.45 t (9.0)				
3″	3.74 dd (2H, 4.8, 1.0)	3.18 m	3.41 m	3.61 m	3.49 t (9.0)				
4″		3.07 m	3.42 m	3.39 m	3.37 t (9.0)				
5″		3.06 m	3.21 m	3.40 m	3.63 m				
6″		3.60 d (9.0)	3.78 d (12.0)	3.83 d (12.0)	4.40 dd (11.4, 2.4)				
		3.38 m	3.66 dd (12.0, 5.4)	3.66 m	4.33 dd (11.4, 6.6)				
1‴				5.55 br s					
2‴				3.98 br s					
3‴					2.05 m				
					1.71 dd (13.6, 10.4)				
4‴				4.18 d (9.6)	4.11 m				
				3.76 d (9.6)					
5‴				3.55 d (10.0)	1.85 dd (13.2, 6.8)				
				3.50 d (10.0)	1.64 m				
6‴									
7‴					6.52 d (15.6)				
8″					7.98 d (15.6)				
9‴									
10‴					5.77 s				
11‴									
12‴					3.80 d (7.2)				
					3.71 d (7.2)				
13‴					0.90 s (3H)				
14‴					1.10 s (3H)				
15‴					2.01 s (3H)				
3-OCH_3	3.82 s (3H)	3.76 s (3H)	3.83, s (3H)	3.81 s (3H)	3.84 s (3H)				
5-OCH_3	3.82 s (3H)		3.83 s (3H)						
3′-OCH_3	3.90 s (3H)	3.77 s (3H)	3.91 s (3H)	3.89 s (3H)	3.90 s (3H)				

Recorded in [a]CD_3OD; in [b]DMSO-d_6; in [c]D_2O.

2.5. Alkaline hydrolyzation of 5

Compound **5** (10 mg) was mixed with MeOH (2.0 mL), *N*,*N*-dimethylformamide (2.0 mL), and 1 M LiOH (2.0 mL) and stirred overnight at room temperature. The mixture was then neutralized with 1 M HCl to determine the absolute configuration of the sugar moiety [6].

2.6. Determination of the absolute configurations of sugar groups

Compounds **2–6** (2.0 mg each) were hydrolyzed with 1 M HCl (1 mL) at 100 °C for 2 h and then extracted with EtOAc (5.0 mL) three times. The H_2O layer was dried, and the residue or sugar standard was dissolved in pyridine (0.5 mL). L-Cysteine methyl ester hydrochloride (2.0 mg) was then added and heated at 60 °C for 2 h. Next, *o*-tolyl isothiocyanate (2.0 μL) was added and further heated at 60 °C for 2 h. The mixture was directly analyzed by HPLC (Agilent 1200) at room temperature. A Diamonsil C-18 HPLC column (5 μm, 150 mm × 4.6 mm; Dikma Technologies Inc., CA, USA) was used for each sample. The samples were eluted isocratically with CH_3CN–H_2O (25:75) at a flow rate of 0.8 mL/min. The wavelength of detection was 250 nm. The retention times of D-glucose (11.56 min), L-glucose (10.69 min), and D-apiose (18.74 min) were compared with those of the reaction mixtures. As the retention times of the sugar derivatives from the compounds were very similar to those of the sugar standards, the types and absolute configurations of the sugars were confirmed [7].

2.7. Cell culture

RAW264.7 cells were purchased from the cell bank of the Chinese Academy of Science and cultured in RPMI-1640 (Invitrogen, Carlsbad,

Table 2
^{13}C NMR spectroscopic data (100 MHz) of compounds **1–8.**

N_O.	1[a]	2[b]	3[a]	4[a]	5[a]	N_O.	6[c]	7[a]	8[a]
1	139.8	132.4	140.1	137.8	138.4	1	123.3		
2	103.9	110.3	104.6	111.1	111.3	2	158.1	161.4	163.2
3	154.9	147.6	154.6	150.7	151.1	3	117.9	113.8	136.0
4	136.5	146.4	135.9	147.8	147.6	4	137.2	161.1	139.6
5	154.9	115.3	154.6	117.3	118.4	5	125.4	117.9	112.2
6	103.9	118.6	104.6	119.3	119.1	6	134.0	149.6	148.6
7	88.9	87.1	89.0	89.0	89.0	7	171.9	162.9	161.1
8	55.8	53.2	55.6	55.5	55.6	1′	102.0	32.5	28.7
9	65.1	62.9	65.1	65.0	65.2	2′	82.7	32.1	31.6
1′	137.3	133.4	132.7	132.6	132.6	3′	78.0	61.8	62.2
2′	112.8	112.1	112.4	112.3	112.3	4′	71.9		
3′	145.4	146.7	145.7	145.7	145.7	5′	78.8		
4′	148.9	143.0	149.5	149.5	149.5	6′	63.3		
5′	129.5	128.6	130.1	130.3	130.1	1″	112.9		67.2
6′	116.7	115.7	116.7	116.7	116.8	2″	79.6		31.8
7′	75.5	82.1	133.9	134.0	134.1	3″	81.7		20.3
8′	77.6	75.4	125.0	124.9	124.9	4″	76.3		14.1
9′	64.4	61.9	72.7	72.7	72.5	5″	66.4		
10′			71.2	71.2	66.7	7-OCH_3	55.7		
11′			33.1	33.1	15.6				
12′			20.5	20.5					
13′			14.4	14.4					
1″	62.2	104.3	105.4	101.0	102.9				
2″	84.9	74.3	75.8	78.9	75.0				
3″	62.2	77.0	78.5	77.6	77.9				
4″		69.9	71.5	71.6	72.0				
5″		76.5	77.9	78.2	75.7				
6″		61.0	62.7	62.6	64.2				
1‴				110.4	83.4				
2‴				78.0	87.9				
3‴				81.0	46.2				
4‴				75.6	66.1				
5‴				66.3	44.6				
6‴					49.6				
7‴					135.9				
8‴					131.9				
9‴					152.4				
10‴					118.5				
11‴					167.5				
12‴					77.4				
13‴					16.6				
14‴					19.8				
15‴					21.4				
3-OCH_3	56.7	55.7	57.2	56.6	56.8				
5-OCH_3	56.7		57.2						
3′-OCH_3	56.9	55.6	56.9	56.9	56.9				

Recorded in [a]CD_3OD; in [b]DMSO-d_6; in [c]D_2O.

CA, USA) supplemented with 10% (*v*/v) fetal calf serum (Hyclone, Logan, UT, USA), 1% (v/v) penicillin/streptomycin solution (Sigma-Aldrich, USA), and glutamine (4 mM; Sigma-Aldrich)]. The cells were incubated at 37 °C in 5% CO_2. The medium was changed every three days and passaged at 80% confluence after trypsinization (0.05%, *w*/*v*). The cultured RAW264.7 cells were seeded at a density of 1×10^4 cells/well (100 μL/ well), then LPS (100 ng/mL) (Invitrogen) was added. The negative control (DMSO), positive control (quercetin), and test compounds (**1–8**) were added in triplicate to the wells to obtain a final concentration of 10 μM. The cells were incubated for 18 h, followed by RNA collection for real-time PCR.

2.8. RNA isolation and evaluation of gene expression

The RAW264.7 cells treated with LPS were examined as previously described [3]. Total RNA was extracted using TRIzol reagent (MRC Inc., Cincinnati, OH, USA), followed by DNase digestion and column cleanup with Qiagen mini-columns (Valencia, CA, USA). Reverse transcription was performed with an iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). The following real-time PCR primers (Integrated DNA Technologies, Coralville, IA, USA) were used: GAPDHR sense (CATCT TCCAGGAGCGAGACC) and anti-GAPDHR sense (GAAGGGGCGGAGA TGATGAC); IL-6 sense (CTGCAAGAGACTTCCATCCAG) and IL-6 antisense (AGTGGTATAGACAGGTCTGTTGG). Real-time PCR was performed with SYBR Green on the 7500 Fast Sequence Detection System (Applied Biosystems, Foster City, CA, USA). All the primers for real-time PCR analysis were designed using Primer Express software 2.0.0 (Applied Biosystems), and GAPDHR was used as the normalization reference.

3. Results and discussion

Eight novel compounds (**1–8**) were isolated from the *n*-BuOH fraction of the leaves and twigs of *S. rubriflora* (Fig. 1).

Compound **1** has a molecular formula of $C_{24}H_{32}O_{11}$, as revealed by the positive ion peak at *m/z* 519.1843 $[M + Na]^+$ (calcd. For $C_{24}H_{32}NaO_{11}$, 519.1837). Compound **1** contains hydroxy groups and benzene rings, as evident from the absorption bands at 3315, 1597, 1503, and 1461 cm^{-1} in the FTIR spectrum. The ^{1}H NMR spectrum (Table 1) suggests the presence of two 1,3,4,5-tetrasubstituted benzene rings [δ_H 6.96 (1H, s, H-2′), 6.89 (1H, s, H-6′), 6.74 (2H, s, H-2, 6)], twelve oxygenated aliphatic protons [δ_H 5.61 (1H, d, J = 5.6 Hz, H-7),

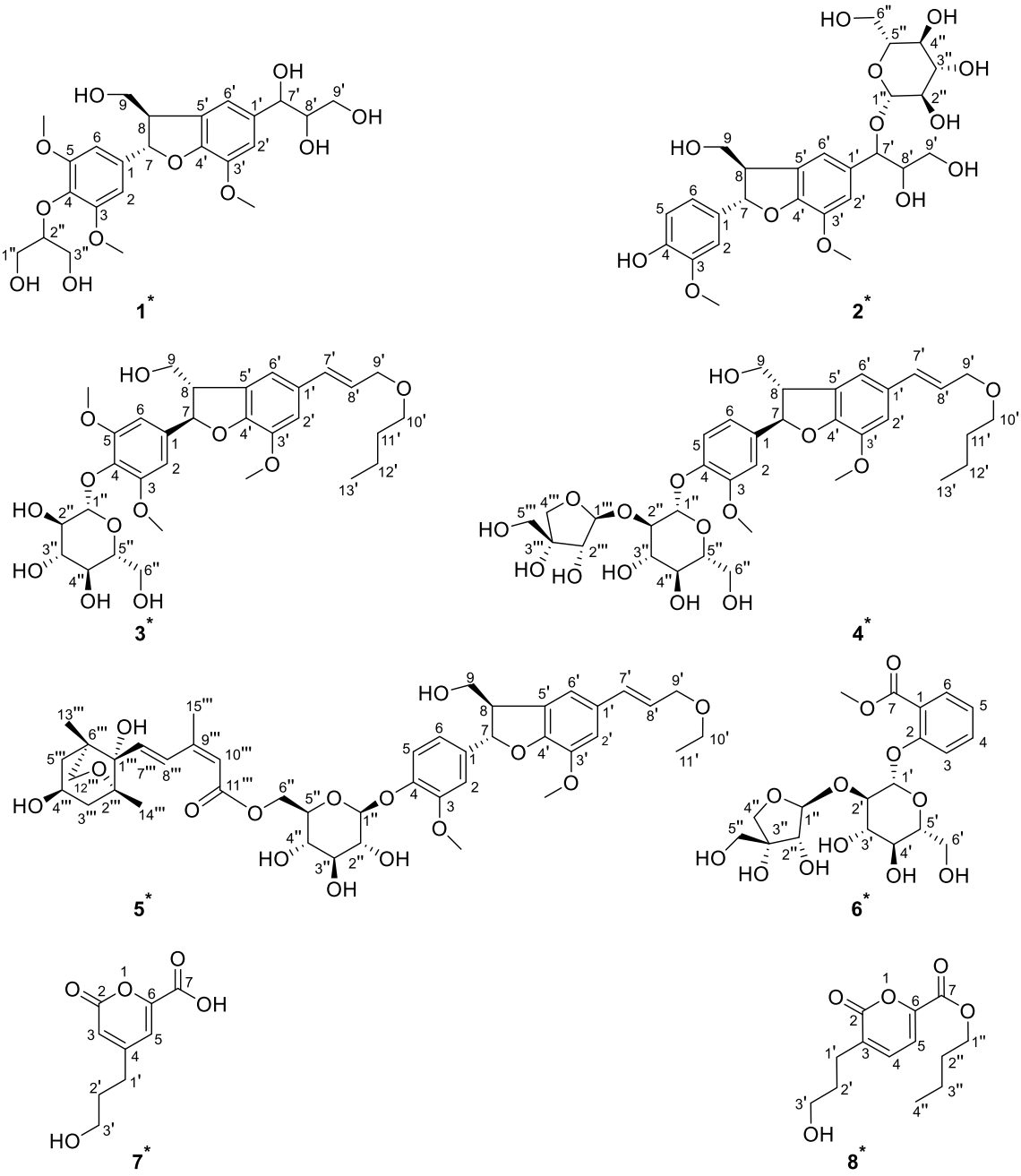

Fig. 1. Structures of compounds **1–8**.

4.58 (1H, d, J = 5.6 Hz, H-7′), 4.00 (1H, t, J = 4.8 Hz, H-2″), 3.88 (1H, m, H-9a), 3.80 (1H, m, H-9b), 3.74 (4H, dd, J = 4.8, 1.0 Hz, H-1″, H-3″), 3.68 (1H, m, H-8′), 3.54 (1H, dd, J = 11.6, 4.4 Hz, H-9″a), 3.38 (1H, dd, J = 11.6, 6.4 Hz, H-9″b)], one non-oxygenated aliphatic proton [δ_H 3.49 (1H, m, H-8)], and three methoxy groups [δ_H 3.90 (3H, s, 3′-OCH_3), 3.82 (6H, s, 3, 5-OCH_3)]. The ^{13}C NMR spectrum of **1** exhibited 24 carbon signals (Table 1), corresponding to twelve aromatic carbons [δ_C 154.9 (C-3, 5), 148.9 (C-4′), 145.4 (C-3′), 139.8 (C-1), 137.3 (C-1′), 136.5 (C-4), 129.5 (C-5′), 116.7 (C-6′), 112.8 (C-2′), 103.9 (C-2, 6)], eight oxygenated aliphatic carbons [δ_C 88.9 (C-7), 84.9 (C-2″), 77.6 (C-8′), 75.5 (C-7′), 65.1 (C-9), 64.4 (C-9′), 62.2 (C-1″, 3″)], one non-oxygenated aliphatic carbon [δ_C 55.8 (C-8)], and three methoxy groups [δ_C 56.9 (3′-OCH_3), 56.7 (3, 5-OCH_3)]. The NMR and HRESIMS data predict that the structure of **1** is similar to that of bennettin [8], except that a propylene glycol group is connected to the hydroxy group at C-4, which was determined by the HMBC correlations of H-2″ (δ_H 4.00)/C-4 (δ_C 136.5). The *trans*-configuration of H-7/H-8 was determined from the coupling constant values (5.6 Hz) and the NOE correlations of H-7 (δ_H 5.61)/H-9 (δ_H 3.88, 3.80) (Fig. 2). The absolute configuration of C-7 and C-8 of **1** was 7*S*, 8*R*, as evident from the positive Cotton effect at 286 nm ($\Delta\varepsilon$ + 0.46) in the CD spectrum, which was in agreement with the data of radulignan [9]. Furthermore, the ^{13}C NMR signals at δ_C 75.5 (C-7′) and δ_C 77.6 (C-8′) in **1** confirmed the *threo* configuration [8]. Consequently, **1** was named as (7*S*,8*R*)-7′,8′-*threo*-4-*O*-1″,3″-dihydroxy-2″-propyl bennettin.

Compound **2** has a molecular formula of $C_{26}H_{34}O_{13}$ ([M + Na]$^+$ as evident from the peak at *m/z* 577.1879 (HRESIMS data). The FTIR spectrum of **2** showed absorption bands corresponding to hydroxyl (3340 cm^{-1}) and aromatic groups (1604, 1494, 1336 cm^{-1}). A comparison of the ^{1}H NMR data of **2** with those of a known compound (meliasendanin D) [10] revealed that the only difference was the presence of an additional glucosyl group [δ_H 4.41 (1H, d, J = 6.4 Hz), 3.60

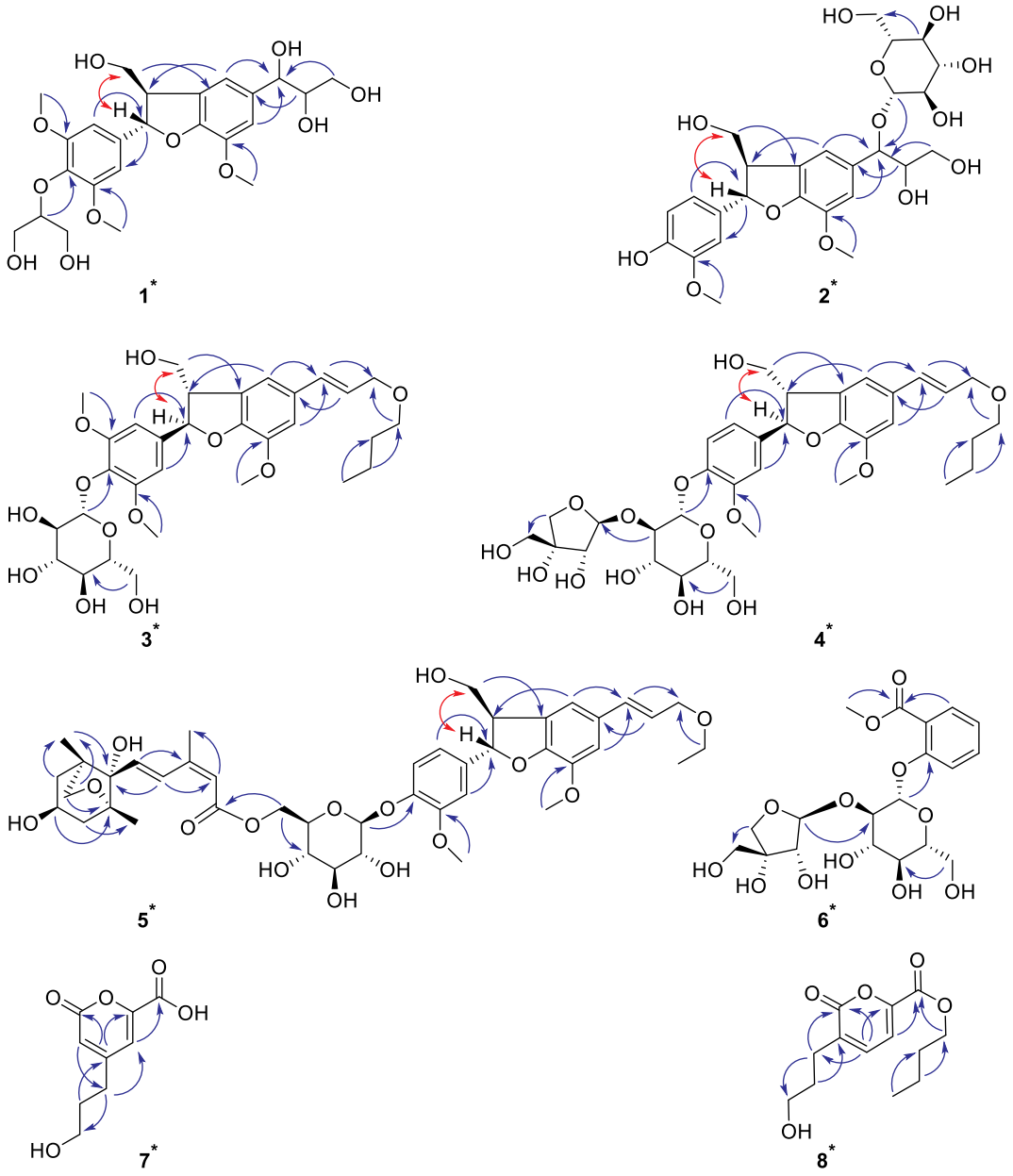

Fig. 2. Key HMBC correlations (blue arrow) and NOEs (red arrow) of compounds **1**–**8**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(d, J = 9.0 Hz), 3.38 (m), 3.18 (m), 3.07 (m), 3.06 (2H, m)] in **2**. The HMBC of the anomeric proton H-1″ (δ_H 4.41, d, J = 6.4 Hz)/C-7′ (δ_C 82.1) confirmed the positions of the glucosyl groups. The *trans*-configuration of H-7/H-8 was determined from the coupling constant values (5.6 Hz) and the NOE correlations of H-7 (δ_H 5.43)/H-9 (δ_H 3.70, 3.62) (Fig. 2). The absolute configuration of **2** was (7*S*,8*R*), as determined from the positive Cotton effect at 286 nm ($\Delta\varepsilon$ + 0.87) in the CD spectrum. This configuration was identical to that of **1** [9]. To further confirm the relative configuration of C-7′ and C-8′ in **2**, its aglycone (**2a**) was obtained by its enzymatic hydrolysis [5]. The *threo* configuration was determined from the chemical shifts of C-7′ and C-8′ (δ_C 75.3 and δ_C 78.2) in **2a** [8]. These findings suggest that the structure of **2a** is identical to that of the known compound, meliasendanin D [10]. HPLC analysis after the acid hydrolysis and derivatization of **2** revealed that the glucosyl residue was in D- configuration [7]. Thus, compound **2** was named as (7*S*,8*R*)-7′,8′-*threo*-meliasendanin D 7′-*O*-*β*-D-glucopyranoside.

The molecular formula of **3** was determined to be $C_{31}H_{42}O_{12}$ from the positive ion peak at m/z 629.2562 $[M + Na]^+$ (HRESIMS data). The absorption bands at 3391, 1598, 1501, and 1462 cm^{-1} in the FTIR spectrum indicated the presence of hydroxyl and phenyl groups and an olefinic bond in the compound. The ^1H and ^{13}C NMR spectra suggest the presence of benzofuran lignan skeleton, as evident from the signals corresponding to two 1,3,4,5-tetrasubstituted benzene rings [δ_H 6.97 (2H, s, H-2′, 6′), 6.74 (2H, s, H-2, 6); δ_C 154.6 (C-3, 5), 149.5 (C-4′), 145.7 (C-3′), 140.1 (C-1), 135.9 (C-4), 132.7 (C-1′), 130.1 (C-5′), 116.7 (C-6′), 112.4 (C-2′), 104.6 (C-2, 6)], one methylene and two methine groups [δ_H 5.60 (d, J = 6.0 Hz, H-7), 3.89 (m, H-9a), 3.81 (m, H-9b), 3.48 (m, H-8); δ_C 89.0 (C-7), 65.1 (C-9), 55.6 (C-8)], and three methoxy groups [δ_H 3.91 (3H, s, 3′-OCH_3), 3.83 (6H, s, 3, 5-OCH_3), δ_C 57.2 (3, 5-OCH_3), 56.9 (3′-OCH_3)]. The other ^1H and ^{13}C NMR signals can be attributed to one butoxylpropenyl group [δ_H 6.57 (1H, d, J = 16.0 Hz, H-7′), 6.20 (1H, dt, J = 16.0, 6.0 Hz, H-8′), 4.11 (2H, d, J = 6.0 Hz, H-9′), 3.49 (2H, t, J = 6.4 Hz, H-10′), 1.60 (2H, m, H-11′), 1.42 (2H, m, H-

12′), 0.95 (3H, t, J = 7.6 Hz, H-13′); δ_C 133.9 (C-7′), 125.0 (C-8′), 72.7 (C-9′), 71.2 (C-10′), 33.1 (C-11′), 20.5 (C-12′), 14.4 (C-13′)] and one glucosyl group [δ_H 4.89 (1H, d, J = 7.2 Hz, H-1″), 3.78 (1H, d, J = 12.0 Hz, H-6″a), 3.66 (1H, dd, J = 12.0, 5.4 Hz, H-6″b), 3.49 (1H, m, H-2″), 3.42 (1H, m, H-4″), 3.41 (1H, m, H-3″), 3.21 (1H, m, H-5″); δ_C 105.4 (C-1″), 78.5 (C-3″), 77.9 (C-5″), 75.8 (C-2″), 71.5 (C-4″), 62.7 (C-6″)]. The planar structure of **3** is very similar to that of the known compound (7*S*,8*R*)-5-methoxydehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside [11], except for the presence of an extra butyl group in **3**. The HMBC of H-10′ (δ_H 3.49)/C-9′ (δ_C 72.7) and H-1″ (δ_H 4.89)/C-4 (δ_C 135.9) indicated the attachment of the butyl group to the hydroxy group at C-9′ and the glucosyl group at C-4. The *trans*-configuration of H-7/H-8 in **3** was identical to that of **2**, as evident from the coupling constant values (6.0 Hz) and the NOE correlations of H-7 and H-9 (Fig. 2). The absolute configuration of **3** was (7*R*,8*S*), as determined from the negative Cotton effect at 275 nm ($\Delta\varepsilon$ − 2.04) in the CD spectrum, which was opposite to that of **1** and **2**. Acid hydrolysis of **3** with 1 M HCl afforded monosaccharides, and HPLC analysis of its derivatives revealed a D configuration [7]. Therefore, **3** was named as (7*R*,8*S*)-9′-*O*-butyl-5-methoxydehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside.

Compound **4** has a molecular formula of $C_{35}H_{48}O_{15}$, as determined from the negative ion peak at m/z 707.2919, $[M - H]^-$ (HRESIMS data). Absorption bands at 1597, 1513, and 1462 cm^{-1} in the FTIR spectrum indicated the presence of aromatic rings, while the absorption band at 3342 cm^{-1} suggested the presence of hydroxyl group. The NMR data (Tables 1 and 2) of **4** were similar to those of **3**, except for the absence of a methoxy group from the benzene ring and the presence of an additional apiosyl group in **4**. This could be deduced from the ^1H, ^{13}C, and HSQC NMR data and the correlations of H-2″ (δ_H 3.72) with C-1‴ (δ_C 110.4) in the HMBC experiment. The absolute configuration of **4** was (7*R*,8*S*), as determined from the coupling constant values (6.0 Hz), NOE correlations (Fig. 2), and CD spectrum (282 nm, $\Delta\varepsilon$ − 4.59). HPLC analysis after acid hydrolysis and derivatization of **4** revealed that the sugar residue was composed of D-glucose and D-apiose, respectively. Therefore, **4** was named as (7*R*,8*S*)-9′-*O*-butyl-dehydrodiconiferyl alcohol 4-*O*-*β*-D-apiofuranosyl-(1 → 2)-*β*-D-glucopyranoside.

Compound **5** has a molecular formula of $C_{43}H_{56}O_{15}$, as determined from the peak at m/z 835.3522 $[M + Na]^+$ (HRESIMS). The FTIR spectrum indicates the presence of hydroxy (3388 cm^{-1}), carbonyl (1702 cm^{-1}), and aromatic functional (1602, 1511, 1460 cm^{-1}) groups. The ^1H and ^{13}C NMR spectral data suggest that the difference between **5** and **4** is the presence of a dihydrophaseic group instead of an apiosyl group in **5**, and an ethoxyl group at C-9′ in **5** replacing the butoxyl group of **4**. The HMBC correlation between H-10′ (δ_H 3.57) and C-9′ (δ_C 72.5) confirmed the presence of ethoxyl group at C-9′. The ester linkage between the dihydrophaseic moiety bound to the glucosyl moiety was determined by the HMBC correlation of H-6″ (δ_H 4.40) and C-11‴ (δ_C 167.5). The absolute configuration of **5** was determined to be 7*S*,8*R* from the coupling constant values (5.6 Hz), NOE correlations (Fig. 2), and the CD spectrum (293 nm, $\Delta\varepsilon$ + 1.58) [10]. HPLC analysis after basic hydrolysis, acid hydrolysis, and derivatization revealed that the glucosyl moiety of **5** was in D configuration [7]. Based on these findings, **5** was named as (7*S*,8*R*)-9′-*O*-ethyl-dehydrodiconiferyl alcohol 4-*O*-*β*-D-{6″-*O*-[(1‴*S*,2‴*R*,4‴*S*,6‴*R*,7‴*E*,9‴*Z*)-dihydrophaseic acyl]}-glucopyranoside.

Compound **6** has a molecular formula of $C_{28}H_{32}O_{10}$, as deduced from the peak at m/z 469.1313 $[M + Na]^+$ (HRESIMS data). The FTIR spectrum of **6** indicated the presence of hydroxy (3345 cm^{-1}), carbonyl (1715 cm^{-1}), and phenyl groups (1601, 1491, 1452 cm^{-1}). The ^1H and ^{13}C NMR data revealed the presence of an *ortho*-disubstituted benzene ring [δ_H 7.78 (1H, br d, J = 7.8 Hz, H-6), 7.61 (1H, td, J = 7.8, 1.2 Hz, H-4), 7.26 (1H, br d, J = 7.8 Hz, H-3), 7.19 (1H, td, J = 7.8, 1.2 Hz, H-5); δ_C 158.1 (C-2), 137.2 (C-4), 134.0 (C-6), 125.4 (C-5), 123.3 (C-1), 117.9 (C-3)], a methoxycarbonyl group [δ_H 3.95 (3H, s, 7-OCH_3); δ_C 171.9 (C-7), 55.7 (7-OCH_3)], a glucosyl moiety [δ_H 5.27 (1H, d, J = 7.2 Hz, H-1′), 3.94 (1H, d, J = 12.6 Hz, H-6'a), 3.78 (1H, dd, J = 12.6, 5.4 Hz, H-6'b), 3.74 (1H, m, H-2′), 3.73 (1H, m, H-4′), 3.65 (1H, m, H-5′), 3.57 (1H, t, J = 8.4 Hz, H-3′); δ_C 102.0 (C-1′), 82.7 (C-2′), 78.8 (C-5′), 78.0 (C-3′), 71.9 (C-4′), 63.3 (C-6′)], and an apiosyl moiety [δ_H 5.37 (1H, d, J = 3.0 Hz, H-1″), 4.02 (1H, td, J = 3.0 Hz, H-2″), 3.89 (1H, d, J = 10.0 Hz, H-4″a), 3.73 (1H, d, J = 10.0 Hz, H-4″b), 3.59 (2H, s, H-5″); δ_C 112.9 (C-1″), 81.7 (C-3″), 79.6 (C-2″), 76.3 (C-4″), 66.4 (C-5″)]. Comparing the NMR data of **6** with those of a known compound, canthoside A [12], indicated very similar structures for the two compounds. The only difference was the position of the apiosyl group. The HMBC of H-1″ (δ_H 5.37, d, J = 3.0 Hz)/C-2′ (δ_C 82.7) indicated that an apiosyl residue was connected to the hydroxy group at C-2′ of the glucosyl residue (Fig. 2). HPLC analysis of the derivatives of the acid hydrolysis product revealed that an absolute configuration of D for glucosyl and apiosyl units [7]. Thus, compound **6** was named as methyl benzoate 2-*O*-*β*-D-apiofuranosyl-(1 → 2)-*β*-D-glucopyranoside.

Compound **7** showed a quasi-molecular ion peak at m/z 197.0455 $[M - H]^-$ in the HRESI mass spectrum, corresponding to the molecular formula $C_9H_9O_5$. The FTIR spectrum showed absorption bands for hydroxy (3397 cm^{-1}), carbonyl (1731 cm^{-1}), and olefinic (1644, 1562, 1438 cm^{-1}) groups. Analysis of the ^1H NMR spectrum (Table 1) suggested the presence of two meta-coupled aromatic protons [δ_H 7.18 (1H, d, J = 1.6 Hz) and 6.41 (1H, d, J = 1.6 Hz)] and a group of hydroxypropyl signals [δ_H 3.61 (2H, t, J = 6.4 Hz), 2.65 (2H, t, J = 8.0 Hz), and 1.84 (2H, m)]. The ^{13}C NMR data of **7** revealed the presence of nine carbon resonances (Table 2), corresponding to two carbonyl groups (δ_C 162.9, 161.4), four olefinic carbon atoms (δ_C 161.1, 149.6, 117.9, 113.8), and one hydroxypropyl group (δ_C 61.8, 32.5, 32.1). The HMBC of H-1′ (δ_H 2.65)/C-3 (δ_C 113.8) and C-5 (δ_C 117.9) and C-3′ (δ_C 61.8) and H-5 (δ_H 6.41)/C-7 (162.9) confirmed that the hydroxypropyl and carboxyl groups were at C-4 and C-6 of the 2-oxo-2*H*-pyran ring, respectively. Thus, compound **7** was named as 4-(3-hydroxypropyl)-2-oxo-2*H*-pyran-6-carboxylic acid.

Compound **8** showed a peak at m/z 255.1221 $[M + H]^+$ in the HRESI mass spectrum, corresponding to the molecular formula $C_{13}H_{19}O_5$. The molecular weight was 56 Da more than that of **7**. Comparison of the ^1H NMR spectrum of **8** with that of **7** revealed the presence of an extra butyl group in **8**, which accounted for its additional 56 Da as compared with **7**. The ^1H NMR spectrum also indicated the presence of two aromatic *ortho*-coupling doublets [δ_H 7.43 (1H, d, J = 6.8 Hz), 7.15 (1H, d, J = 6.8 Hz)], which was different from the meta-coupling protons in **7**. The differences in the ^1H NMR spectra of **8** and **7** were confirmed by the HMBC correlations of H-1″ (δ_H 4.32)/C-7 (δ_C 161.1), C-3″ (δ_C 20.3), H-1′ (δ_H 2.56)/C-2 (δ_C 163.2), C-4 (δ_C 139.6), and C-3′ (δ_C 62.2). Hence, **8** was named as butyl 3-(3-hydroxypropyl)-2-oxo-2*H*-pyran-6-carboxylate.

Compounds **1**–**8** were evaluated for their inhibitions of IL-6 expression induced by LPS in RAW 264.7 cells. The results revealed that two lignans (**1** and **5**) and a pyran derivative (**7**) at the concentration of 10 μM could inhibit the expression of IL-6 up to 55%, 52%, and 72%. The positive control, quercetin, inhibited the same by 75%. (See Fig. 3.)

4. Conclusions

Leaves and twigs of *S. rubriflora* are commonly used as folk medicines to treat inflammatory pathophysiological conditions such as rheumatism, tracheitis, and cough. Three compounds (**1**, **5**, **7**) at the concentration of 10 μM exhibited certain inhibitory effects on the production of IL-6 with inhibitions of 55, 52 and 72%, respectively, while the positive control quercetin possessed an inhibition of 75%. The results showed that lignans without sugar residues (**1** and **5**) could inhibit the IL-6 expression more effectively than the glycosides of lignans (**2**–**4**). These findings indicated that the three compounds have potential to become new anti-inflammatory agents.

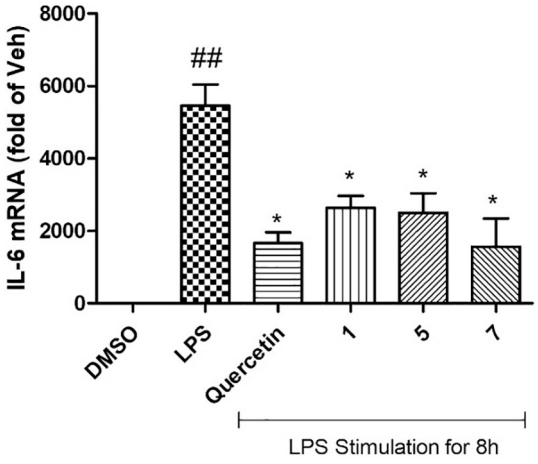

Fig. 3. Effects of compounds **1**, **5**, **7** and the positive control (quercetin) on IL-6 expression induced by LPS. The bars represent the mean ± SD ($n = 3$) of three independent experiments ($^{*}P < .05$ compared to LPS group, $^{\#\#}P < .01$ compared to DMSO group).

Funding

This study was supported by the CAMS Innovation Fund for Medical Sciences (CIFMS) (2016-I2M-1-010), Fund of Jiansheng Fresh Herb Medicine Research, and Drug Innovation Major Project (2018ZX09711-001-001-001 and 2018ZX09711-001-001-003).

Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.104417.

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