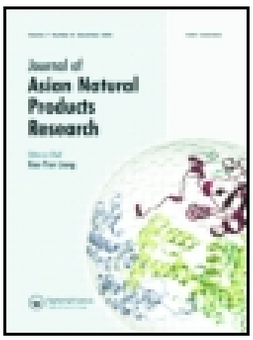

Journal of Asian Natural Products Research

ISSN: 1028-6020 (Print) 1477-2213 (Online) Journal homepage: http://www.tandfonline.com/loi/ganp20

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To cite this article: Yan-Fei Liu, Guo-Ru Shi, Xin Wang, Chun-Lei Zhang, Yan Wang, Ruo-Yun Chen & De-Quan Yu (2016): Nine new compounds from the whole plants of Rehmannia chingii, Journal of Asian Natural Products Research, DOI: 10.1080/10286020.2016.1173680

To link to this article: http://dx.doi.org/10.1080/10286020.2016.1173680

JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH, 2016
http://dx.doi.org/10.1080/10286020.2016.1173680

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Nine new compounds from the whole plants of *Rehmannia chingii*

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ABSTRACT

Nine new compounds, together with 16 known analogs, were isolated from the whole plants of *Rehmannia chingii*. The structures of compounds **1**–**9** were elucidated on the basis of their spectroscopic data and chemical evidence. In addition, the new compounds were tested for their hepatoprotective activities against APAP-induced HepG2 cell damage and their ability to inhibit LPS-induced nitric oxide production in the murine microglia BV2 cell line. Compounds **2** and **5** exhibited pronounced hepatoprotective activities against APAP-induced HepG2 cell damage at a concentration of 10 μM, and compounds **4** and **9** showed moderate inhibitory activity against microglial inflammation factor with IC_{50} values of 3.51 and 7.11 μM, respectively.

ARTICLE HISTORY
Received 2 February 2016
Accepted 30 March 2016

KEYWORDS
Scrophulariaceae; *Rehmannia chingii*; iridoid; ionone glucoside; hepatoprotective

1. Introduction

The plants of the genus *Rehmannia* (Scrophulariaceae) consist of six species distributed, mainly in the eastern Asia. *Rehmannia glutinasa* is the most popular one. Its rhizome is known as "Dihuang" and used for the recovery of a variety of disorders in traditional Chinese medicine [1]. This genus is a rich source of diverse iridoid glycosides, ionone glycosides, and phenethyl alcohol glycosides [2–5]. *Rehmannia chingii* H. L. Li, an endemic species, is mainly distributed in the Zhejiang Province in China, which was used as folk medicine for the treatment of fever and bleeding. However, the chemical and pharmacological study of this plant has not yet been carried out thoroughly. In our search for bioactive constituents of *R. chingii*, nine new compounds were isolated from the aqueous extract of the whole plants of *R. chingii*. Herein, we report the isolation, structural elucidation, and biological assays of nine new compounds (**1**–**9**).

2. Results and discussion

Compound **1** was obtained as an amorphous powder with a specific rotation of $[\alpha]_D^{20} - 134.5$. Its molecular formula, $C_9H_{14}O_5$, was deduced from HRESIMS (*m/z* 201.0763 $[M–H]^-$).

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The IR spectrum showed absorption bands for hydroxy (3360 cm^{-1}) group. The ^1H NMR spectrum of **1** displayed resonances for a hemiacetal proton at δ 5.34, two oxygen-bearing methylene groups at δ 3.62 and 3.83–3.84, and δ 4.13–4.14 and 4.28–4.29, two oxygenated methine protons at δ 3.67 and 4.39–4.40, and three other aliphatic proton signals in the range δ 1.42–2.76. In consistent with these observations, the ^{13}C NMR spectrum showed nine carbon resonances comprising a hemiacetal carbon, three methylenes (two oxygenated), three methines (two oxygenated), and a pair of olefinic carbons at δ 137.5 and 138.2. The ^1H–^1H COSY correlations from H-4α to H-5 and H_2-3, H-6–H-5, and H-7 established the presence of –$CH_2CH_2CHCHCH$–, which were similar to those reported for rehmaglutin A [5]. Analysis of the ^1H–^1H COSY, HSQC and HMBC spectra led to the complete assignments of the proton and carbon signals in compound **1** (Table 1). The ROESY correlations of H-1/ H-6 and H-3α, H-6/H-7 and H-4α, H-5/H-3β and H-4β indicated that H-1, H-5, H-6, H-7 are successively α-, β-, α-, and α-oriented. Therefore, compound **1** (rehmachinin A) was characterized.

Compound **2** was assigned a molecular formula of $C_9H_{12}O_3$ from its HRESIMS (*m/z* 191.0677 $[M+Na]^+$). The ^1H NMR spectrum of **2** displayed resonances for an olefinic proton signal at δ 5.68–5.69, an oxygenated methylene group at δ 3.56 and 3.87, a singlet methyl at δ 1.73 (3H, s), and several methylene and methine groups in the range δ 2.16–3.05. The ^{13}C NMR spectrum showed nine carbon resonances comprising a methyl, three methylenes (two oxygenated), a methine, an oxygenated quaternary carbon, and a carbonyl carbon. A trisubstituted olefinic C-atoms were apparent at δ 131.7 and 138.9. The signals for five protons in the upfield region were assigned to the fragment –CH_2CHCH_2– by ^1H–^1H COSY. The spectroscopic data of compound **2** were similar to those of rehmalgutin C [5]. The structure of this compound was determined by the analysis of its 2D-NMR spectrum including HSQC and HMBC. The ROESY correlation between H-5 and H-1 suggested a *cis* orientation. The structure of **2** (rehmachinin B) was therefore elucidated as shown in the Figure 1.

The molecular formula of compound **3** was determined as $C_9H_{11}NO_2$ from the HRESIMS (*m/z* 166.0863 $[M+H]^+$). The ^1H NMR spectrum of **3** showed three olefinic proton signals at δ 8.46, 8.36, and 7.31, an oxygenated methine group at δ 4.28, a singlet methyl at δ_H 1.46 (3H, s). Its ^{13}C NMR spectrum showed nine carbon resonances comprising a methyl, a methylene, an oxygenated methine, an oxygenated quaternary carbon, and five olefinic carbons (δ 151.3, 149.2, 145.4, 145.3, 122.1). The spectroscopic data of compound **3** were similar to those of salviadiginine A [6]. The structure of this compound was confirmed by detailed analysis of the 2D-NMR spectra (^1H–^1H COSY, HSQC, and HMBC). The ROESY correlation of CH_3-8/H-7 indicated that the H-7 and CH_3-8 are both α-oriented. The CD spectrum of **3** exhibited similar Cotton effect (positive at 263 nm) to that of salviadiginine A [6], indicating that the asymmetric centers of **3** at C-7 and C-8 are S and R-configured, respectively. Hence, the structure of compound **3** (rehmachinin C) was determined.

Compound **4** was isolated as amorphous powder, and its molecular formula was established as $C_{10}H_{16}O_3$ on the basis of the HRESIMS (*m/z* 183.1026 $[M-H]^-$). The ^1H NMR spectrum of **4** showed an olefinic proton signal at δ 5.59 (1H, s), an olefinic methyl at δ 1.62 (3H, s), two singlet methyls at δ 0.98 and 0.92 (each 3H, s). Its ^{13}C NMR spectrum showed 10 carbon resonances comprising 3 methyls, 2 methylenes, 2 quaternary carbons (one oxygenated), 2 olefinic carbons (δ 127.1, 134.5), and 1 carboxylic group at δ 178.3. Analysis of the 2D-NMR (^1H–^1H COSY, HSQC, and HMBC) data led to the structure of compound **4**. On the basis of the allylic quasi-axial hydrogen rule analysis, a positive Cotton

Table 1. ^{1}H NMR (600 MHz for **1**–**3**, and at 500 MHz for **4**.) and ^{13}C NMR (150 MHz for **1**–**3**, and at 125.0 MHz for **4**.) spectral data for **1**–**4** (CD_3OD).

	1		**2**		**3**		**4**	
Position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	5.34 s	96.3	3.87 d (12.0) 3.56 d (12.0)	64.4	8.46 s	145.4		37.2
2*α*							2.04–2.05 m	34.3
2*β*							1.39–1.40 m	
3*α*	3.62 ddd (11.4, 4.2, 1.2)	59.4		179.6	8.36 d (4.8)	149.2	2.08–2.09 m	23.4
3*β*	3.83–3.84 m						2.03–2.04 m	
4*α*	1.42 dd (12.0, 4.2)	32.9	2.36 dd (18.0,5.4)	38.7	7.31 dd (4.8, 0.6)	122.1	5.59 br s	127.1
4*β*	2.11 ddd (12.0, 6.0, 1.2)		2.94 dd (18.0,10.2)					
5	2.76–2.77 m	46.0	3.04–3.05 m	39.1		151.3		134.5
6*α*	3.67 dd (4.2, 1.2)	79.5	2.15–2.16 m	38.6	2.75 ddd (16.8, 6.6, 0.6)	38.6		81.7
6*β*			2.67–2.68 m		3.26 dd (16.8, 6.6)			
7*α*	4.39–4.40 m	74.5	5.68–5.69 m	131.7	4.28 t (6.6)	82.2		178.3
7*β*								
8		138.2		138.9		82.0	0.98 s	23.6
9		137.5		102.8		145.3	0.92 s	25.2
10a	4.28–4.29 m	56.7	1.73 s	12.4	1.46 s	21.5	1.62 d (1.0)	18.8
10b	4.13–4.14 m							

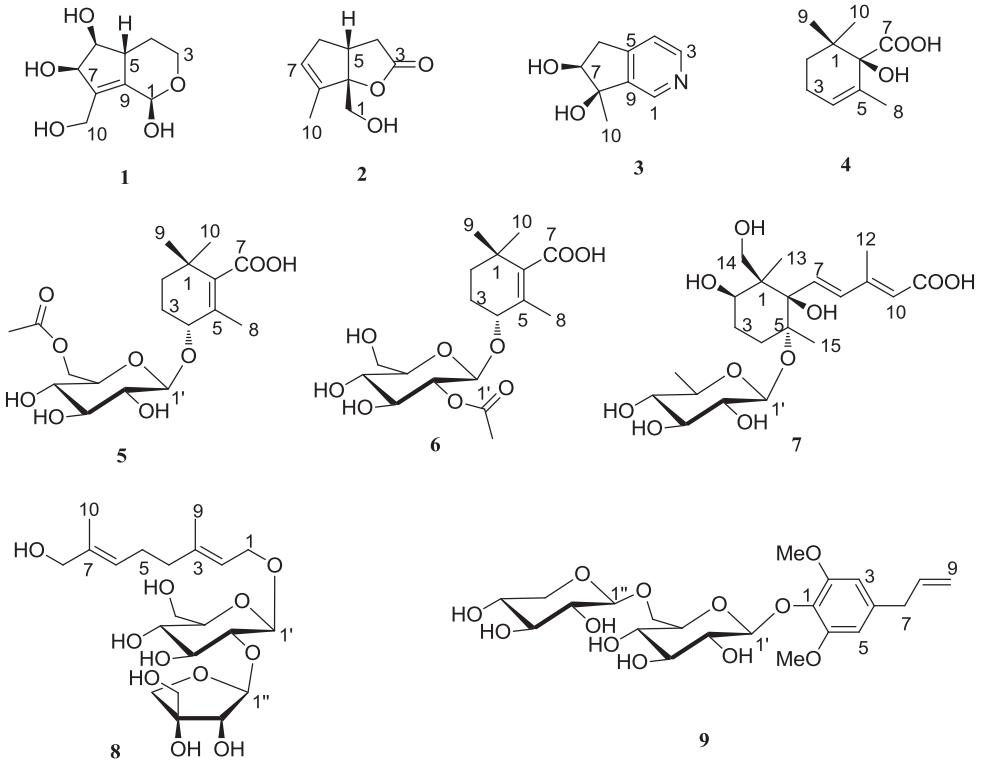

Figure 1. Structures of compounds **1–9**.

effect at 218 nm in the CD spectrum of **4** indicated that it had a 6*R* configuration [7]. From these data, compound **4** was characterized as (6*R*)1-hydroxy-2,6,6-trimethylcyclohex-2-enecarboxylic acid.

Compound **5**, a white powder, had the molecular formula $C_{18}H_{28}O_9$ from its positive-mode HRESIMS (*m/z* 387.1658 $[M-H]^-$). The IR spectrum showed absorption bands due to hydroxyl group (3382 cm^{-1}) and an α, β-unsaturated carboxyl moiety (1715 and 1654 cm^{-1}). The 1D-NMR data revealed the presence of two tertiary methyl groups at δ 1.14 (6H, s), an olefinic methyl at δ 1.78 (3H, s), a tetrasubstituted olefin moiety, a carboxylic group, and a glucopyranosyl moiety, which were in good agreement with those of rehmapicroside [7]. The only evident difference was that **5** showed resonances due to an additional acetyl group located at C-6′ of the glucopyranosyl moiety confirmed by HMBC correlation of H-1′ with C-4 (δ 75.4) and H-6′ with the carbonyl at δ 172.7. The CD spectrum of **5** exhibited a similar Cotton effect (positive at 232 nm) as that of rehmapicroside [7], indicating *R* configuration, which was also verified by allylic quasi-axial hydrogen rule analysis [3]. Acid hydrolysis of **5** afforded glucose, which was identified by TLC comparison with authentic sample. The β-anomeric configuration in glucopyranosyl moiety was judged from its large $^3J_{H1,H2}$ coupling constant (J = 7.5 Hz). On the basis of the above data, compound **5** (6′-acetyl rehmapicroside) was characterized as shown.

Compound **6** was found to have the same molecular formula as **5** ($C_{18}H_{28}O_9$), provided by its HRESIMS (*m/z* 387.1660 $[M-H]^-$). The spectroscopic data of **6** (Table 2) were very similar to those of **5** except that the position of the acetyl group was located at C-2′ in

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data for **5**–**7** (CD_3OD).

	5		**6**		**7**	
Position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		34.0		34.0		45.7
2*α*	1.74–1.75 m	34.9	1.56–1.57 m	34.6	3.89–3.90 m	72.5
2*β*	1.40–1.41 m		1.36–1.37 m			
3*α*	1.90–1.91 m	25.4	1.82–1.83 m	25.6	1.55–1.57 m	25.4
3*β*	1.89–1.90 m		1.76–1.77 m		2.41–2.43 m	
4	4.05 t (4.5)	75.4	4.02–4.03 m	75.8	1.65–1.67 m	27.0
					1.96–1.98 m	
5		130.9		130.0		83.8
6		142.0		142.8		80.6
7		174.5		175.3	6.62 d (16.0)	138.2
8	1.14 s	27.5	1.11 s	27.4	6.35 d (16.0)	134.4
9	1.14 s	28.9	1.08 s	29.1		152.8
10	1.78 s	18.4	1.78 s	18.6	5.77 s	120.5
11						171.8
12					2.27 s	14.3
13					1.21 s	19.1
14a					4.07 d (11.0)	65.5
14b					3.20–3.21 m	
15					1.15 s	21.2
1′	4.38 d (7.5)	102.0	4.52 d (8.0)	100.3	4.44 d (8.0)	98.0
2′	3.19 dd (9.0, 7.5)	74.8	4.66 dd (9.5, 8.0)	75.4	3.20–3.21 m	75.6
3′	3.39 t (9.0)	77.9	3.54–3.55 m	76.2	3.30–3.31 m	78.8
4′	3.32–3.34 m	71.8	3.37–3.38 m	71.7	3.24–3.25 m	72.6
5′	3.46–3.48 m	75.1	3.29–3.30 m	78.0	3.01 t (9.0)	77.0
6′a	4.42 dd (12.0, 2.0)	64.7	3.87 dd (12.0, 2.0)	62.7	1.22 d (6.0)	18.4
6′b	4.26 dd (12.0, 6.0)		3.69 dd (12.0, 6.0)			
***CH*$_3$CO**	2.07 s	20.7	2.07 s	21.1		
CH$_3$***CO***		172.7		171.7		

6 instead of at C-6′ in **5**. This was confirmed by HMBC correlation of H-2′ (*δ* 4.66) with the ester carbonyl (*δ* 171.7). The CD spectrum of **6** exhibited a similar Cotton effect (positive at 234 nm) as that of rehmapicroside [7], indicating *R* configuration. Consequently, compound **6** was characterized as 2′-acetyl rehmapicroside.

Compound **7** exhibited a $[M-H]^-$ ion peak at *m/z* 445.2077 in its HRESIMS, corresponding to the molecular formula $C_{21}H_{34}O_{10}$. The ^{13}C NMR spectrum of **7** showed 21 signals, of which 15 could be attributed to a norcarotenoid moiety [8], and rest 6 to a quinovopyranosyl unit. The ^{1}H NMR spectrum exhibited signals for an olefinic proton at δ_H 5.77 (1H, s), *trans*-olefinic protons at *δ* 6.35 and 6.62 (each 1H, d, *J* = 16.0 Hz), an olefinic methyl at *δ* 2.27 (3H, s), and an anomeric proton at *δ* 4.44 (1H, d, *J* = 8.0 Hz, H-1′), which were in good agreement with those of jiocarotenoside A_1 except for an additional secondary hydroxyl group located at C-2 [8]. This was confirmed by HMBC correlations of H-2 with C-6, H-13 with C-2, together with an ^{1}H–^{1}H COSY correlation between H-2 (*δ* 3.89–3.90) and H_2-3 (*δ* 1.55–1.57, 2.41–2.43). The ROESY correlations of H-2/H-3α and H-13, H-15/H-3β and H-14 (*δ* 3.20–3.21, 4.07), and H-13/H-7 indicated that the hydroxy groups at C-2, C-6, and the methyl group at C-5 are β-oriented. The CD spectrum of **7** exhibited similar Cotton effects (positive at 219 nm and negative at 261 nm) to those of *sec*-hydroxyaeginetic acid [8], indicating that C-2, C-5, C-6 are *R* configurated. On the basis of the above evidence, compound **7** was characterized as *sec*-hydroxyjiocarotenoside A_1.

Compound **8** exhibited a $[M+Na]^+$ ion peak at *m/z* 487.2161 in its HRESIMS, corresponding to the molecular formula $C_{21}H_{36}O_{11}$. The ^{1}H NMR spectrum displayed resonances

for two olefinic methyls at δ 1.70 and 1.66 (each 3H, s), two oxygen-bearing methylenes [δ 4.23 (1H, dd, J = 12.0, 7.2 Hz) and δ 4.33–4.34 (1H, m)] and [δ 3.93 (2H, m)], two trisubsituted olefinic proton signals at δ 5.37–5.38 and 5.40–5.41, two anomeric proton signals [δ 4.32 (1H, d, J = 7.8 Hz, H-1′), 5.35 (1H, d, J = 1.2 Hz, H-1″)]. In agreenment with these observations, the ^{13}C NMR spectrum showed 21 carbon resonances comprising 6 for a glucopyranosyl unit, 5 for a apiofuranosyl unit [9], and 10 for 8-hydroxygeraniol [10]. The position of the glucopyranosyl group was confirmed by HMBC correlation of H-1′ with C-1 and H-1 with C-1′. ^{1}H–^{1}H COSY correlation of H-1′/H-2′ together with HMBC correlation of H-2′ with C-1″ verified the location of the apiofuranosyl group. Acid hydrolysis of **8** afforded D-glucose and D-apiose, which were identified by TLC and optical rotation comparison with authentic samples. Accordingly, compound **8** was characterized as 8-hydroxygeraniol 1-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside.

Compound **9** was assigned a molecular formula of $C_{22}H_{32}O_{12}$ from its HRESIMS (*m/z* 533.1869 $[M+COOH]^-$). The IR spectrum showed absorption bands for hydroxyl groups (3402 cm^{-1}) and aromatic ring (1594, 1506 cm^{-1}). The ^{1}H NMR spectrum indicated the presence of two methoxyl groups [δ 3.84 (6H, s)], an allyl group [δ 3.35 (2H, d, J = 6.6 Hz), δ 5.06–5.11 (2H, m), δ 5.97 (1H, ddt, J = 16.8, 10.2, 6.6 Hz)] [11], two aromatic protons [δ 6.54 (2H, s)], and two anomeric proton signals [δ 4.77 (1H, d, J = 7.8 Hz, H-1′), 4.25 (1H, d, J = 7.2 Hz, H-1″)]. The ^{13}C NMR spectrum showed 22 carbon signals including 6 for glucopyranosyl unit, 5 for xylpyranosyl unit, 11 for 4-allyl-2, 6-dimethoxyphenol aglycon [9,11]. The position of the glucopyranosyl unit was confirmed by HMBC correlation of H-1′ (δ 4.77) with C-1 (δ 138.6) and that of the xylpyranosyl group by a cross-peak between H-1″ (δ 4.25) and C-6′ (δ 69.2). Acid hydrolysis of **9** afforded glucose and xylose, respectively. Thus, compound **9** was characterized as 4-allyl-2,6-dimethoxyphenol 1-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside.

The known compounds were identified as jiofuran (**10**) [4], rehmaglutin A (**11**) [5], rehmaglutin B (**12**) [5], rehmaglutin D (**13**) [5], rehmapicrogenin (**14**) [12], frehmaglutin E (**15**) [3], rehmapicroside (**16**) [7], rehmapicroside methyl ester (**17**) [7], trihydroxy-β-ionone (**18**) [12], dihydroxy-β-ionone (**19**) [12], jiocarotenoside A_1 (**20**) [8], aeginetic acid 5-*O*-β-D-quinovoside (**21**) [13], aeginetic acid (**22**) [12], kankanoside P (**23**) [14], kankanoside O (**24**) [14], and 4-allyl-2-methoxyphenyl 6-*O*-β-D-apiosyl (1→6)-β-D-glucoside (**25**) [15], by NMR analysis and comparison with those reported.

Compounds **1**–**9** were tested for their cytotoxicity against five human tumor cell lines, A549 (human lung epithelial cell line), HT-29 (human colon cancer cell line), Bel-7402 (human hepatoma cell line), BGC-823 (human gastric cancer cell line), and A2780 (human ovarian cancer cell line). However, all were inactive for all cell lines used (IC_{50} > 10 μM is defined as "inactive"). These compounds were also evaluated for their inhibitory activity against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in murine microglia BV2 cells. As shown in Table 3, compounds **4** and **9** exhibited moderate anti-inflammatory activities (IC_{50} values < 10 μM), and no influence on cell viability was observed using the MTT method. Their hepatoprotective activity against *N*-acetyl-*p*-aminophenol (APAP)-induced toxicity in HepG2 (human hepatocellular liver carcinoma cell line) cells was tested, using the hepatoprotective activity drug bicyclol as positive control [16]. As shown in Table 4, compounds **2** and **5** exhibited pronounced hepatoprotective activity.

Table 3. Inhibitory effects of compounds **4** and **9** against LPS-induced NO production in murine microglia BV2 cells.[a]

Compound	IC_{50} (μM)	Cell viability[b]
4	3.51 ± 0.42	94.4 ± 1.8
9	7.11 ± 0.59	83.1 ± 1.4
curcumin[c]	0.52 ± 0.08	93.4 ± 3.0

[a]Results are reported as means ± SD based on three independent experiments.
[b]Cell viability is expressed as a percentage (%) of the LPS-only treatment group.
[c]Positive control.

Table 4. Hepatoprotective effects of compounds **2** and **5** (10 μM) against APAP-induced toxicity in HepG2 cells.[a]

Compound	cell survival rate (% of normal)
normal	100 ± 1.9
control	58.5 ± 3.7
bicyclol	66.5 ± 1.8[b]
2	70.1 ± 1.3[b]
5	71.3 ± 2.2[b]

[a]Results are expressed as means ± SD ($n = 3$; for normal and control, $n = 6$). bicyclol was used as positive control (10 μM).
[b]$p < 0.001$.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a JASCO P-2000 polarimeter, and UV spectra with a JASCO V-650 spectrophotometer (JASCO Corporation, Tokyo, Japan). IR spectra were recorded on a Nicolet 5700 spectrometer (Thermo Eletron Scientific Instruments Corp.) by an FT-IR microscope transmission method. NMR measurements were performed on Bruker AV500-III and Bruker AV600 IIIHD spectrometers using TMS as an internal reference (Bruker Biospin Corporation, Fallanden, Switzerland) in methanol-d_4. HRESIMS were obtained using an Agilent 1100 series LC/MSD ion trap mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (GE), and ODS (50 μm, YMC, Kyoto, Japan) were used for column chromatography. Analytical thin-layer chromatography (TLC) was carried out with GF254 plates (Qingdao Marine Chemical Factory). Spots were visualized by spraying with 10% H_2SO_4 in 95% EtOH followed by heating.

3.2. Plant material

Whole plants of *Rehmannia chingii* were collected in Hangzhou, Zhejiang Province, China, in July 2013, and identified by associate professor Lin Ma (Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College). A voucher specimen (ID-S-2577) has been deposited at the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing.

3.3. Extraction and isolation

The air-dried whole plants (15 kg) of *R. chingii* were extracted two times with H_2O (3 × 45 L) under reflux (2 h each). The combined extracts were concentrated under reduced pressure

to dryness. The residue was suspended in H_2O and applied to a Diaion HP20 column eluted gradiently with EtOH/H_2O (0:100, 45:55, and 95:5, v/v) to afford three fractions. After removing the solvent, the EtOH/H_2O (45:55) eluate (297 g) was separated over silica gel eluted with $CHCl_3$/MeOH (6:1 to 0:1, v/v) to afford three fractions (F_1–F_3) based on TLC analysis. The EtOH/H_2O (95:5) eluate (251 g) was separated over a silica gel column, eluted gradiently with methanol in chloroform (0–100%) to afford four fractions (F_4–F_7). Fraction F_1 (91.3 g) was chromatographed on a reversed-phase C_{18} silica gel column (7.5 × 47 cm) eluted with a MeOH/H_2O (1:99–70:30) gradient to give subfractions F_{1-1}–F_{1-27}. Separation of fraction F_{1-1} (55 mg) over reversed-phase silica gel (1.8 × 35 cm) eluted with MeOH/H_2O (2:98) yielded **1** (7 mg) and **3** (7 mg). Fractions F_{1-22} (25 mg) and F_{1-24} (30 mg) were subjected separately to separation over Sephadex LH-20 column (MeOH-H_2O, 1:1) to yield **5** (10 mg) from F_{1-22} and **6** (11 mg) from F_{1-23}. Fraction F_{1-26} was further separated repeatedly over Sephadex LH-20 column to afford **4** (10 mg). Fraction F_4 (77.3 g) was separated over a reversed-phase C_{18} silica gel column (7.5 × 47 cm) and eluted with MeOH/H_2O (5:95 to 80:20) gradient to yield subfractions F_{4-1}–F_{4-42}. The separation of fraction F_{4-10} (33 mg) over silica gel (EtOAc-EtOH-H_2O, 20:2:1; 15:2:1, 10:2:1) gave **2** (7 mg). Eluting with a stepwise gradient of MeOH/H_2O (5:95 to 50:50), Fraction F_5 (58.5 g) was fractioned over a reversed-phase C_{18} silica gel column (7.5 × 47 cm), to give subfractions F_{5-1}–F_{5-26}. Fraction F_{5-17} (202 mg) was purified through repeated silica gel column chromatography (EtOAc–EtOH–H_2O, 15:2:1; 10:2:1, 7:2:1) to give **7** (11 mg). Fraction F_6 (51.7 g) was separated by a reversed-phase C_{18} silica gel column (7.5 × 47 cm) eluted gradiently with MeOH/H_2O (10:90–90:10) to yield subfractions F_{6-1}–F_{6-42}. Fraction F_{6-19} (1.2 g) was separated over silica gel (EtOAc–EtOH–H_2O, 12:2:1; 9:2:1; 7:2:1) and Sephadex LH-20 (MeOH/H_2O, 1:1) to yield **8** (10 mg) and **9** (12 mg).

3.3.1. Rehmachinin A (1)

Amorphous powder; $[\alpha]_D^{20}$ − 134.5 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.23) nm; IR ν_{max} 3360, 2922, 2502, 2067, 1682, 1395, 1186, 1041 cm^{-1}; ^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz) spectral data, see Table 1; HR-ESI-MS: *m/z* 201.0763 $[M-H]^-$ (calcd for $C_9H_{13}O_5$, 201.0768).

3.3.2. Rehmachinin B (2)

Amorphous powder; $[\alpha]_D^{20}$ − 5.4 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.14) nm; CD (MeOH) $\Delta\varepsilon_{222nm}$ + 0.43, $\Delta\varepsilon_{264.5nm}$ + 0.39; IR ν_{max} 3406, 2922, 2868, 1750, 1647, 1449, 1215, 1011, 956 cm^{-1}; ^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz) spectral data, see Table 1; HR-ESI-MS: *m/z* 191.0677 $[M+Na]^+$ (calcd for $C_9H_{12}O_3Na$, 191.0679).

3.3.3. Rehmachinin C (3)

Amorphous powder; $[\alpha]_D^{20}$ − 51.9 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.94), 257 (2.95) nm; CD (MeOH) $\Delta\varepsilon_{215.5nm}$ − 4.62, $\Delta\varepsilon_{263.5nm}$ + 3.08; IR ν_{max} 3358, 3020, 2719, 1610, 1445, 1364, 1100, 1065, 1030 cm^{-1}; ^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz) spectral data, see Table 1; HR-ESI-MS: *m/z* 166.0863 $[M+H]^+$ (calcd for $C_9H_{12}NO_2$, 166.0863).

Table 5. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectral data for **8** and **9**(CD_3OD).

	8		**9**	
Position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	4.23 dd (12.0, 7.2) 4.33 m	66.2		138.6
2	5.37–5.38 m	121.7		154.1
3		141.7	6.54, s	107.4
4	2.11–2.12 m, 2.20–2.21 m	40.3		134.4
5	1.70–1.71 m, 2.21–2.22 m	26.9	6.54, s	107.4
6	5.40–5.41 m	126.1		154.1
7		136.2	3.35 d (6.6)	41.4
8	3.93 s	68.9	5.97 ddt (16.8, 10.2, 6.6)	138.6
9	1.70 s	16.5	5.06–5.07, m, 5.10–5.11, m	116.2
10	1.66 s	13.8	3.84 s	57.0
1′	4.32 d (7.8)	101.6	4.77, d (7.8)	104.9
2′	3.36 dd (9.0, 7.8)	78.5	*a*	75.4
3′	3.47 t (8.4)	78.6	*a*	77.9
4′	3.30 t (9.0)	71.7	*a*	71.3
5′	3.22–3.24 m	77.8	*a*	77.3
6′a	3.86 dd (12.0, 2.4)	62.7	*a*	69.2
6′b	3.67 dd (12.0, 6.0)			
1″	5.35 d (1.2)	110.4	4.25, d (7.2)	105.6
2″	3.94 d (1.2)	77.8	*a*	74.7
3″		80.8	*a*	77.4
4″	3.72 d (10.2)	75.4	*a*	71.1
	4.03 d (10.2)			
5″	3.61–3.63 m	66.3	*a*	66.7

a: Overlapped between *δ* 3.22–3.48.

3.3.4. 1-Hydroxy-2,6,6-trimethylcyclohex-2-enecarboxylic acid (4)

Amorphous powder; $[\alpha]_D^{20}$ − 21.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.09) nm; CD (MeOH) $\Delta\varepsilon_{218.5nm}$ + 2.06; IR ν_{max} 3438, 2973, 2546, 2229, 2061, 1714, 1610, 1366, 1249, 1186, 1072 cm^{-1}; ^{1}H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz) spectral data, see Table 1; HR-ESI-MS: *m/z* 183.1026 $[M-H]^+$ (calcd for $C_{10}H_{15}O_3$, 183.1027).

3.3.5. 6′-Acetylrehmapicroside B (5)

Amorphous powder, $[\alpha]_D^{20}$ − 13.9 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.42) nm; CD (MeOH) $\Delta\varepsilon_{232nm}$ + 0.82; IR ν_{max} 3382, 2963, 2931, 1715, 1654, 1546, 1367, 1249, 1080, 1043 cm^{-1}; ^{1}H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz) spectral data, see Table 2; HR-ESI-MS: *m/z* 387.1658 $[M-H]^-$ (calcd for $C_{18}H_{27}O_9$, 387.1661).

3.3.6. 2′-Acetylrehmapicroside (6)

Amorphous powder; $[\alpha]_D^{20}$ − 9.0 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.31) nm; CD (MeOH) $\Delta\varepsilon_{234nm}$ + 0.67; IR ν_{max} 3382, 2966, 2933, 1716, 1635, 1376, 1249, 1078 cm^{-1}; ^{1}H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz) spectral data, see Table 2; HR-ESI-MS: *m/z* 387.1660 $[M-H]^-$ (calcd for $C_{18}H_{27}O_9$, 387.1661).

3.3.7. s-Hydroxyjiocarotenoside A_1 (7)

Amorphous powder; $[\alpha]_D^{20}$ − 87.2 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 261 (4.59) nm; CD (MeOH) $\Delta\varepsilon_{219nm}$ + 1.40, $\Delta\varepsilon_{260.5nm}$ − 3.74; IR ν_{max} 3375, 2975, 2936, 1685, 1611, 1445, 1244, 1069 cm^{-1}; ^{1}H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz) spectral data, see Table 2; HR-ESI-MS: *m/z* 445.2077 $[M-H]^-$ (calcd for $C_{21}H_{33}O_{10}$, 445.2079).

3.3.8. 8-Hydroxygeraniol 1-O-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside (8)

White amorphous powder; $[\alpha]_D^{20}$ − 9.7 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.39) nm; IR ν_{max} 3366, 2922, 1668, 1380, 1074, 1041 cm^{-1}; ^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz) spectral data, see Table 5; HR-ESI-MS: *m/z* 487.2161 $[M+Na]^+$ (calcd for $C_{21}H_{36}O_{11}Na$, 487.2150).

3.3.9. 4-Allyl-2,6-dimethoxyphenol 1-O-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (9)

White amorphous powder; $[\alpha]_D^{20}$ − 60.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.53) nm; IR ν_{max} 3570, 3402, 2919, 2848, 1594, 1506, 1462, 1241, 1042 cm^{-1}; ^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz) spectral data, see Table 5; HR-ESI-MS: *m/z* 533.1869 $[M+COOH]^-$ (calcd for $C_{23}H_{33}O_{14}$, 533.1876).

3.4. Acid hydrolysis of 5–9

Each compound (5 mg) was individually refluxed in 6% HCl (3.0 ml) at 80 °C for 2 h. Each reaction mixture was extracted with $CHCl_3$ (3 × 6 ml), and the H_2O phase was dried using a N_2 stream. The residues were separately subjected to column chromatography over silica gel with EtOAc–EtOH–H_2O (6:2:1) as eluent to yield glucose (1.90 mg) from **5**, $[\alpha]_D^{20}$ + 46.2 (*c* 0.10, H_2O); glucose (1.12 mg) from **6**, $[\alpha]_D^{20}$ + 42.7 (*c* 0.07, H_2O); quinovose (0.34 mg) from **7**, $[\alpha]_D^{20}$ + 13.1 (*c* 0.02, H_2O); glucose (0.82 mg) and apiose (0.66 mg) from **8**, $[\alpha]_D^{20}$ + 40.3 (*c* 0.05, H_2O) and $[\alpha]_D^{20}$ + 4.2 (*c* 0.04, H_2O); glucose (0.69 mg) and xylose (0.89 mg) from **9**, $[\alpha]_D^{20}$ + 39.6 (*c* 0.04, H_2O), and $[\alpha]_D^{20}$ + 17.8 (*c* 0.05, H_2O), respectively. The sugars were confirmed as D-glucose, D-quinovose, D-apiose, and D-xylose by comparison with an authentic sample on TLC (EtOAc–EtOH–H_2O, 7:2:1, R_f 0.07, R_f 0.09, R_f 0.22, and R_f 0.26) and by measuring its optical rotation as shown above.

3.5. Cytotoxicity assay

Compounds **1**–**9** were tested for cytotoxicity against HCT-8 (human colon carcinoma), Bel-7402 (human liver carcinoma), BGC-823 (human stomach carcinoma), A549 (human lung carcinoma), and A2780 (human ovarian carcinoma) by means of a MTT method described in the literature [17].

3.5.1. Inhibitory effects on NO production in LPS-activated microglia

Compounds **1**–**9** were tested for their ability to inhibit LPS-activated NO production in the BV2 cell line. Curcumin was used as the positive control [16].

3.5.2. Hepatoprotective activity assay

The hepatoprotective effects of compounds **1**–**9** were determined by a MTT colorimetric assay in HepG2 hepatoma cells [18].

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The authors gratefully acknowledge grants from the National Natural Science Foundation of China [81403076]; the State Key Laboratory of Bioactive Substance and Function of Natural Medicines [GTZC201201]; Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

References

[1] Chinese Pharmacopoeia Commission, *Pharmacopoeia of the People's Republic of China*, (China Medical Science Press, Beijing, 2010), p. 115.
[2] Y.F. Liu, D. Liang, H. Luo, Z.Y. Hao, Y. Wang, C.L. Zhang, Q.J. Zhang, R.Y. Chen, and D.Q. Yu, *J. Nat. Prod.* **75**, 1625 (2012).
[3] Y.F. Liu, D. Liang, H. Luo, Z.Y. Hao, Y. Wang, C.L. Zhang, G. Ni, R.Y. Chen, and D.Q. Yu, *J. Asian Nat. Prod. Res.* **16**, 11 (2014).
[4] T. Morota, H. Nishimura, H. Sasaki, M. Chin (Chen Zhengxiong), K. Sugama, and H. Mitsuhashi, *Phytochemistry*. **28**, 2385 (1989).
[5] I. Kitagawa, Y. Fukuda, T. Taniyama, and M. Yoshikawa, *Chem. Pharm. Bull.* **34**, 1399 (1986).
[6] S.J. Wu, C.H. Huang, Y.Y. Chan, Y.R. Liao, T.L. Hwang, and T.S. Wu, *Int. J. Mol. Sci.* **15**, 11566 (2014).
[7] M. Yoshikawa, Y. Fukuda, T. Taniyama, and I. Kitagawa, *Chem. Pharm. Bull.* **44**, 41 (1996).
[8] H. Sasaki, H. Nishimura, T. Morota, T. Katsuhara, M. Chin (Chen Zhengxiong), and H. Mitsuhashi, *Phytochemistry*. **30**, 1639 (1991).
[9] H. Nishimura, H. Sasaki, T. Morota, M. Chin (Chen Zhengxiong), and H. Mitsuhashi, *Phytochemistry*. **29**, 3303 (1990).
[10] K. Koromi and J. Komatsu, *Yakugaku zasshi*. **103**, 508 (1983).
[11] T. Miyase, M. Kuroyanagi, T. Noro, A. Ueno, and S. Fukushima, *Chem. Pharm. Bull.* **33**, 4445 (1985).
[12] H. Sasaki, T. Morota, H. Nishimura, T. Ogino, T. Katsuhara, K. Sugama, M. Chin (Chen Zhengxiong), and H. Mitsuhashi, *Phytochemistry*. **30**, 1997 (1991).
[13] S.L. Liu, J.S. Kim, R.J. Choi, Y.S. Kim, J.H. Lee, and S.S. Kang, *Chem. Pharm. Bull.* **59**, 742 (2011).
[14] T. Morikawa, Y. Pan, K. Ninomiya, K. Imura, D. Yuan, M. Yoshikawa, T. Hayakawa, and O. Muraoka, *Chem. Pharm. Bull.* **58**, 1403 (2010).
[15] K. Machida, Y. Nakano, and M. Kikuchi, *Phytochemistry*. **30**, 2013 (1991).
[16] Y. Zhang, Y.B. Liu, Y. Li, S.G. Ma, L. Li, J. Qu, D. Zhang, X.G. Chen, J.D. Jiang, and S.S. Yu, *J. Nat. Prod.* **76**, 1058 (2013).
[17] C.L. Zhang, Y. Wang, Y.F. Liu, D. Liang, Z.Y. Hao, H. Luo, Q.J. Zhang, G.R. Shi, R.Y. Chen, Z.Y. Cao, and D.Q. Yu, *Tetrahedron*. **71**, 5579 (2015).
[18] Y. Li, D.M. Zhang, J.B. Li, S.S. Yu, Y. Li, and Y.M. Luo, *J. Nat. Prod.* **69**, 616 (2006).