Note

New Iridoids from *Scrophularia ningpoensis*

Qing-juan Ma, Li Han,* Yu Mu, Pei-pei Guan, Hui Lei, Zhan-you Wang, and Xue-shi Huang*

Institute of Microbial Pharmaceuticals, College of Life and Health Sciences, Northeastern University; Wenhua Road 3–11, Shenyang 110819, P. R. China.

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Five new compounds including five iridoids (1–5) and six known compounds were isolated from the rhizomes of *Scrophularia ningpoensis*. Their structures were determined by extensive NMR and IR, MS spectroscopic data analyses. The anti-inflammatory, antibacterial, antifungal, and cytotoxic activities of the isolated compounds were evaluated. Compound 11 exhibited significant inhibitory effects on lipopolysaccharide-induced nitric oxide production in RAW264.7 macrophage cells.

Key words *Scrophularia ningpoensis*; iridoid; pyrrolidin-2-one; anti-inflammatory

Scrophularia ningpoensis HEMS., belonging to the family Scrophulariaceae, is a kind of perennial herbal medicine widely distributed in China. Its dried roots have been used as an important traditional Chinese medicine named “Xuan Shen” for the treatment of bacteria, atopy, pimple, tonsillitis, angina, encephalitis, and various inflammation diseases.^{1,2)} Previous phytochemical studies on *S. ningpoensis* showed that iridoids^{3–8)} and phenylpropanoid glycosides^{7–9)} are the main chemical constituents of *S. ningpoensis*. Moreover, saponins¹⁰⁾ and alkaloids¹¹⁾ were also isolated from *S. ningpoensis*. Iridoids as the main chemical constituents are assumed to be the predominant active pharmaceutical ingredients of *S. ningpoensis*.^{1,12,13)}

As our search for unique and bioactive compounds from traditional Chinese medicine, the chemical constituents of the dried roots of *S. ningpoensis* were investigated. In present study, we reported the isolation and structural elucidation of compounds 1–11. The anti-inflammatory, cytotoxic and antimicrobial activities of isolated compounds were evaluated.

Results and Discussion

Seventy percent EtOH extract from the dried roots of *S. ningpoensis* was subjected to silica gel, Sephadex LH-20, and octadecyl silica (ODS) column chromatography to afford 11 pure compounds. They were three new iridoids, 1 β ,6 β -dimethoxy-dihydrocatalpolgenin (1), ningpogeniridoid (2), 1-ethoxyl-3-hydroxy-2,3-seconingpogenin (4), two pairs of new inseparable isomers, 1 β -hydroxy-6 β -methoxy-dihydrocatalpolgenin (3a)/1 α -hydroxy-6 β -methoxy-dihydrocatalpolgenin (3b) and ningpopyrrosides A/B (5a/b) together with six known compounds, 5-(methoxymethyl)-1H-pyrrole-2-carbaldehyde (6),¹⁴⁾ 5-methoxypyrrolidin-2-one (7),^{15,16)} ningpogenin (8),^{3,17)} pedicularis lactone (9),¹⁸⁾ iridolactone (10),¹⁹⁾ and scrophularianine A (11)¹¹⁾ (Fig. 1). All compounds were identified based on detailed spectroscopic data analysis. Among them, compounds 5 and 7 were 5-hydroxypyrrolidin-2-ones, which had a rare five-membered heterocyclic lactam with a hemiaminal group in natural source including plants and microorganisms. To our best knowledge, this is the first report of the isolation of 5-hydroxypyrrolidin-2-ones from *S. ningpoensis*.

Compound 1 was acquired as a colorless oil and its molecular formula was deduced as C₁₁H₁₈O₅ on the basis of

high resolution-electrospray ionization (HR-ESI)-MS data (*m/z* 253.1045 [M+Na]⁺, Calcd C₁₁H₁₈NaO₅, 253.1052). The ¹H-NMR data of 1 (Table 1) presented five methine protons including three oxygenated methines at δ 4.10 (1H, d, *J*=8.6 Hz), 3.78 (1H, brd, *J*=9.2 Hz), 3.62 (1H, brs) and two aliphatic methines at δ 1.97 (1H, t, *J*=8.3 Hz), 1.92 (1H, brq, *J*=7.5 Hz); three methylenes at δ 3.76 (1H, dd, *J*=12.1, 5.5 Hz), 3.41 (1H, td, *J*=12.1, 2.2 Hz), δ 3.70 (1H, dd, *J*=12.8, 5.3 Hz), 3.49 (1H, dd, *J*=12.8, 6.8 Hz), and δ 1.60 (1H, m), 1.40 (1H, brd, *J*=14.1 Hz); two methoxyls at δ 3.37 (3H, s), 3.35 (3H, s). In addition, an active proton was observed at δ 4.46 (1H, dd, *J*=6.8, 5.3 Hz). ¹³C-NMR data gave eleven carbons, except for the carbons corresponding to the proton signals, one more carbon without directly bonded proton was presented at δ 65.4 (C-8) with the help of heteronuclear single quantum coherence (HSQC) experiment. The ¹H- and ¹³C-NMR data of 1 was very similar to those of dihydrocatalpolgenin²⁰⁾ except for two more methoxyls appeared in 1. Heteronuclear multiple bond connectivity (HMBC) correlations from 1-OMe (δ 3.35) to C-1 (δ 102.0), from 6-OMe (δ 3.37) to C-6 (δ 80.9), from H-1 (δ 4.10) to 1-OMe (δ 55.8), and from H-6 (δ 3.78) to 6-OMe (δ 57.3) indicated the two methoxyls were located at C-1 and C-6, respectively. The relative configuration of 1 was determined by analysis of the coupling constants between H-1 and H-9 (*J*_{H1–H9}=8.6 Hz), H-5 and H-9 (*J*_{H5–H9}=8.3 Hz), H-5 and H-6 (*J*_{H5–H6}=9.2 Hz), and H-6 and H-7 (*J*_{H6–H7} about 0 Hz) suggesting 1 possessed the same relative configuration as dihydrocatalpolgenin.^{21,22)} The nuclear Overhauser effect (NOE) correlations between 10-OH (δ 4.46) and H-1 (δ 4.10), H-6 (δ 3.78), H-10a (δ 3.70), H-10b (δ 3.49), between H-1 (δ 4.10) and H-7 (δ 3.62), H-10b (δ 3.49), H-3b (δ 3.41), H-9 (δ 1.97), between 1-OMe (δ 3.35) and H-5 (δ 1.92), between 6-OMe (δ 3.37) and H-9 (δ 1.97) also confirmed H-1, H-6, H-7, and hydroxymethyl at C-8 were α -oriented, H-5, H-9, 1-OMe, and 6-OMe were β -oriented (Fig. 2). Thus, the structure of 1 was determined and named 1 β ,6 β -dimethoxy-dihydrocatalpolgenin.

Compound 2 was isolated as a colorless oil and its molecular formula was deduced as C₁₁H₁₈O₅ from HR-ESI-MS and NMR spectroscopic data. The ¹H- and ¹³C-NMR data of 2 were characteristic of an iridoid. Comparing NMR data of 2 (Table 1) with those of a related iridoid, 1 β ,6 β ,7 α ,8 α ,10-pentahydroxy-*cis*-2-oxabicyclo[4.3.0]nonane,²¹⁾ one more olefinic double bond and two additional methoxyls were pre-

* To whom correspondence should be addressed. e-mail: hanli@mail.neu.edu.cn; huangxs@mail.neu.edu.cn

Fig. 1. Structures of Compounds 1–11

Fig. 2. Key NOE Correlations of Compounds 1 and 2

Fig. 3. Key HMBC Correlations of Compounds 2, 4 and 5

Table 1. ^1H -NMR (600 MHz) and ^{13}C -NMR (150 MHz) Data for 1–3 in $\text{DMSO}-d_6$

Position	1		2		3a		3b	
	δ_{C}	δ_{H} mult. (J in Hz)	δ_{C}	δ_{H} mult. (J in Hz)	δ_{C}	δ_{H} mult. (J in Hz)	δ_{C}	δ_{H} mult. (J in Hz)
1	102.0	4.10, d (8.6)	94.9	5.30, s	94.0	4.32, dd (8.2, 6.2)	98.4	5.18, t (4.0)
3	61.4	3.76, dd (12.1, 5.5) 3.41, td (12.1, 2.2)	57.9	3.66, t (11.6) 3.53, dd (11.6, 3.3)	60.3	3.68, dd (11.9, 5.3) 3.40, td (11.9, 2.0)	52.3	3.83, dd (12.5, 2.8) 3.30, td (12.5, 5.5)
4	23.4	1.60, m 1.40, brd (14.1)	32.6	1.97, dd (12.8, 5.9) 1.34, qd (12.8, 4.6)	22.5	1.57, m 1.37, brd (13.9)	21.9	1.66, m 1.47, brd (13.6)
5	35.2	1.92, brq (7.5)	42.8	2.70, m	34.3	1.90, brq (7.5)	31.3	1.76, brq (7.5)
6	80.9	3.78, brd (9.2)	87.6	3.21, t (6.1)	79.8	3.78, brd (8.4)	81.7	3.82, brd (9.0)
7	56.6	3.62, brs	70.8	4.38, brt (6.4)	55.4	3.61, brs	56.7	3.50, brs
8	65.4		138.5		64.5		65.0	
9	42.4	1.97, t (8.3)	133.5		43.0	1.93, brt (8.0)	38.4	2.29, brt (5.5)
10	59.3	3.70, dd (12.8, 5.3) 3.49, dd (12.8, 6.8)	55.5	4.11, dd (13.5, 4.4) 3.49, ddd (13.5, 6.2, 2.9)	58.4	3.77, dd (13.0, 5.3) 3.60, dd (13.0, 7.2)	59.2	3.95, dd (12.5, 6.4) 3.30, dd (12.5, 5.5)
1-OMe	55.8	3.35, s	54.2	3.25, s				
6-OMe	57.3	3.37, s	57.5	3.31, s	56.2	3.36, s	56.3	3.35, s
1-OH					6.71, d (6.1)		6.71, brd (4.0)	
7-OH			4.43, d (7.0)					
10-OH		4.46, dd (6.8, 5.3)	4.72, dd (6.2, 4.0)		4.37, dd (7.2, 5.3)		4.37, t (6.2)	

sented and one methine and one oxygenated quaternary were disappeared in **2**. HMBC correlations between H-1 (δ 5.30), 10-OH (δ 4.72), 7-OH (δ 4.43), H-7 (δ 4.38), H-10 (δ 4.11, 3.49) and C-8 (δ 138.5), between H-7 (δ 4.38), H-10 (δ 4.11, 3.93) and C-9 (δ 133.5), between 1-OMe (δ 3.25) and C-1 (δ 94.9), between 6-OMe (δ 3.31) and C-6 (δ 87.6) showed the olefinic double bond was located at C-8, C-9 and two methoxys were linked with C-1 and C-6, respectively (Fig. 3). The coupling constants of H-5 and H-6 ($J_{\text{H5-H6}}$ =6.1 Hz), H-6 and H-7 ($J_{\text{H6-H7}}$ =6.4 Hz) indicated both of H-5/H-6 and H-6/H-7 were *anti* configurations.²²⁾ NOE correlations observed between

1-OMe (δ 3.25) and H-7 (δ 4.38), H-3 β (δ 3.66), between H-3 β (δ 3.66) and H-5 (δ 2.70) suggested methoxy at C-1 was β configuration (Fig. 2). Thus, the structure of **2** was determined and named ningpogeniridoid.

Compounds **3a** and **b** were obtained as an inseparable mixture of two epimers in a 5:3 ratio according to ^1H -NMR integral signals. The molecular formulas of **3a** and **b** were established as $\text{C}_{10}\text{H}_{16}\text{O}_5$ by HR-ESI-MS. Two sets of signals could be obviously assigned in the ^1H - and ^{13}C -NMR spectra according to their intensity as well as correlation spectroscopy (COSY) and HSQC experiments. The ^1H - and ^{13}C -NMR data

of **3a** and **b** (Table 1) were characteristic of iridoids and close resemblance to dihydrocatalpolgenin.²³⁾ The difference between them was an additional methoxy signal presented both in **3a** (δ 56.2, δ 3.36) and **3b** (δ 56.3, δ 3.35). The methoxyls located at C-6 both in **3a** and **b** were confirmed by HMBC correlations between 6-OMe (δ 3.36 in **3a**)/C-6 (δ 79.8 in **3a**) and 6-OMe (δ 3.35 in **3b**)/C-6 (δ 81.7 in **3b**). Except for the configurations of C-1, the chiral carbons configuration of **3a** and **b** were identical to those of compound **1** and dihydrocatalpolgenin according to the coupling constants and NOE correlations. The larger coupling constant between H-1 and H-9 ($J_{\text{H1-H9}}=8.2\text{ Hz}$) of the major compound **3a** was consistent with a β position of the hemiacetal hydroxy at C-1. While, a small value of $J_{\text{H1-H9}}=4.0\text{ Hz}$ showed the hemiacetal hydroxyl was α configuration in **3b**.^{20,23)} Therefore, the structures were elucidated as 1 β -hydroxy-6 β -methoxy-dihydrocatalpolgenin (**3a**) and 1 α -hydroxy-6 β -methoxy-dihydrocatalpolgenin (**3b**), respectively.

Compound **4** was assigned the molecular formula $\text{C}_{11}\text{H}_{20}\text{O}_4$ by HR-ESI-MS (m/z 239.1259 $[\text{M}+\text{Na}]^+$). The ^1H -NMR data of **4** (Table 2) showed signals for one olefinic proton at δ 5.93 (1H, s), four couples of oxygenated methylenes at δ 4.14 (1H, dd, $J=15.4, 4.4\text{ Hz}$), 3.99 (1H, dd, 15.4, 5.5 Hz), δ 3.50 (1H, m), 3.34 (1H, m), δ 3.48 (1H, m), 3.35 (1H, m), and δ 3.47 (1H, m), 3.43 (1H, m), an oxygenated methine at δ 4.03 (1H, brd, $J=5.7\text{ Hz}$), a pair of methylene at δ 1.61 (1H, m), 1.55 (1H, m), two methines at δ 2.45 (1H, q, $J=6.4\text{ Hz}$) and 2.20 (1H, quint, $J=7.2\text{ Hz}$), and one methyl at δ 1.06 (3H, t, $J=6.0\text{ Hz}$), in addition to three active protons at δ 4.83 (1H, t, $J=5.3\text{ Hz}$), 4.37 (1H, t, $J=5.3\text{ Hz}$), and δ 4.09 (1H, dd, $J=6.4, 3.5\text{ Hz}$). The ^{13}C -NMR spectrum (Table 2) manifested eleven carbons including two olefinic carbons (δ 155.1, 124.9), five oxygenated aliphatic carbons (δ 81.6, 63.6, 61.7, 60.2, 60.1), two methines (δ 48.9, 42.7), one methylene (δ 29.1), and one methyl (δ 16.0) with the aid of HSQC experiment. The NMR data of **4** showed a close similarity to those of a related iridoid, ningpo-

genin,^{3,17)} which also isolated from the same herbal medicine. The difference between **4** and ningpogenin (**8**) were one more ethoxyl and one more hydroxyl arising in **4** on the basis of NMR data and molecular formula. COSY and HMBC correlations deduced **4** was a 2,3-seco-ring ningpogenin derivative with an ethoxyl group. HMBC correlations from H-1' (δ 3.48, 3.35) to C-1 (δ 81.6), from H-1 (δ 4.03) to C-1' (δ 63.6) revealed ethoxy located a C-1 (Fig. 3). The relative configuration of **4** was identical with ningpogenin deduced from the coupling constant of H-1 and H-5 ($J_{\text{H1-H5}}=7.2\text{ Hz}$), H-5 and H-6 ($J_{\text{H5-H6}}=6.4\text{ Hz}$). Consequently, the structure of **4** was determined to be 1-ethoxyl-3-hydroxy-2,3-seconingpogenin.

Compound **5** being as a pair of inseparable stereoisomers (**5a/5b**) were isolated as a mixture. The molecular formulas were established to be $\text{C}_{13}\text{H}_{19}\text{NO}_4$, based on their HR-ESI-MS data as well as ^{13}C -NMR data. The ^1H -NMR and ^{13}C -NMR spectrum (Table 2) of **5a** showed characteristic signals for ningpogenin moiety and a set of more signals containing one carbonyl (δ 177.9), one oxygenated methine (δ 86.6, δ 4.90), two methylenes (δ 28.5, 28.1, δ 2.23/2.02, 2.17/1.85), and an active proton (δ 8.68). The fragment was confirmed as 5-hydroxypyrrolidin-2-one residue based on the COSY, HSQC, and HMBC experiments.^{15,24)} HMBC correlations from H-5' (δ 4.85) to C-9 (δ 66.1) and from H-9 (δ 3.62/3.44) to C-5' (δ 85.8) revealed the connection of the two moieties (Fig. 3), ningpogenin and 5-hydroxypyrrolidin-2-one, as in Fig. 1.

The ^1H - and ^{13}C -NMR spectra of **5** presented another set of signals for **5b**, with a ratio of nearly 1:1 with **5a**. The ^1H - and ^{13}C -NMR data of **5b** were almost identical with **5a**, except for chemical shift changes at H-9 (δ 3.62/3.44), H-5' (δ 4.85), C-7 (δ 149.8) and C-8 (δ 124.8) for **5a**, H-9 (δ 3.45/3.28), H-5' (δ 4.87), C-7 (δ 149.5) and C-8 (δ 125.0) for **5b**, indicating **5b** is a C-1' isomer of **5a**. The HMBC correlations from H-5' (δ 4.87) to C-9 (δ 66.1) and from H-9 (δ 3.45/3.28) to C-5' (δ 85.8) confirmed the structure. Alike **4**, the relative configurations of **5a/b** were established by analysis on the coupling constant

Table 2. ^1H -NMR (600 MHz) and ^{13}C -NMR (150 MHz) Data for **4** and **5** in $\text{DMSO}-d_6$

Position	4		5a/5b	
	δ_{C}	δ_{H} mult. (J in Hz)	δ_{C}	δ_{H} mult. (J in Hz)
1	81.6	4.03, brd (5.7)	86.6	4.90, brd (7.0)
3	60.1	3.47, m 3.43, m	66.7	3.61, m, 3.45, m
4	29.1	1.61, m 1.55, m	28.3	1.74, m, 1.66, m
5	42.7	2.20, quint (7.2)	42.8	2.90, m
6	48.9	2.45, q (6.4)	46.0	2.85, m
7	155.1		149.8/149.5	
8	124.9	5.93, s	124.8/125.0	5.48, d (1.5)
9	61.7	3.50, m	66.1	3.62, m, 3.44, m
		3.34, m		3.45, m, 3.28, dd (9.0, 8.0)
10	60.2	4.14, dd (15.4, 4.4)	59.4	3.98, dd (15.4, 6.8)
		3.99, dd (15.4, 5.5)		3.89, d (15.4)
1'	63.6	3.48, m, 3.35, m, 3.34, m		8.68, s
2'	16.0	1.06, t (6.0)	177.9	
3'			28.5	2.23, m, 2.02, m
4'			28.1	2.17, m, 1.85, m
5'			85.8	4.85, d (7.0)/4.87, d (7.0)
3-OH		4.37, t (5.3)		
9-OH		4.09, dd (6.4, 3.5)		
10-OH		4.83, t (5.3)		4.76, brs

Fig. 4. Effects of Compound **11** on the Inhibition of LPS-Induced NO in RAW264.7 Cell

Cells were treated by LPS with or without different concentrations of compounds, respectively (**11**, 33, 100 μM) for 24 h. Minocycline (50 μM) was used as a positive control. Nitrite–nitrate levels were measured in the medium and expressed as % activity over LPS stimulation. All conditions were run in triplicate, and data show mean $\pm$ S.D. values. * $p < 0.01$ compared to LPS.

values of $J_{\text{H1-H5}} = 7.0 \text{ Hz}$. Even though the $^1\text{H-NMR}$ spectrum didn't give a clear coupling constant of H-5 and H-6, a weak NOE correlation of H-1 (δ 4.90) and H-6 (δ 2.85) could be assigned both *syn* relationships of H-1 and H-5, H-5 and H-6. Therefore, the structures of **5a/b** were determined, and named as ninpopyrrosides A and B.

All of the isolated compounds (**1–11**) were assayed for their anti-inflammatory, antibacterial, antifungal, and cytotoxic activities. Anti-inflammatory activities of isolated compounds were tested by examining their ability to inhibit nitric oxide (NO) production in lipopolysaccharide (LPS)-treated RAW264.7 macrophage cells. Among the tested compounds, only **11** showed a dose-dependent inhibition of NO production at the tested concentrations (11, 33, 100 μM) (Fig. 4). The other compounds show very weak activity with an inhibitory ratio lower than 20% at 100 μM . The cytotoxic activities and antimicrobial activities were also assessed for compounds **1–11**, and no compounds exhibited obvious inhibitory activity.

Experimental

General Experimental Procedures Optical rotations were determined using an AntonPaar MCP200 automatic polarimeter. Ultraviolet spectra were measured using a Beckman Coulter DU 730 nucleic acid/protein analyzer. IR spectra were recorded using a Bruker Tensor 27 FT-IR spectrometer (film). One and two dimensional (1D- and 2D)-NMR spectra were recorded using a Bruker AV-600 spectrometer, δ in ppm rel. to tetramethylsilane (TMS), J in Hz. ESI-MS were measured using an Agilent 1290-6420 triple quadrupole LC-MS spectrometer. HR-ESI-MS were measured using a Bruker micro TOF-Q mass instrument (Bruker Daltonics, Billerica, MA, U.S.A.). Silica gel (100–200 mesh and 200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), Sephadex LH-20 (GE Healthcare Biosciences AB, Uppsala, Sweden), and YMC-GEL ODS-A (S-50 μm , 12 nm) (YMC Co., Ltd., Kyoto, Japan) were used for column chromatography. Biological assays were analyzed using a microplate reader (BioTek Synergy H1, BioTek Instruments, Inc., Vermont, U.S.A.).

Plant Material Dried rhizomes of *S. ningpoensis* were purchased from the Anguo Huirui Pharmaceutical Co., Ltd.,

Hebei Province, China, in August 2014 (batch No. 141101) and were identified by Professor Jinhui Wang from Shenyang Pharmaceutical University, Shenyang, China. A voucher specimen (No. 141101) was deposited at the Institute of Microbial Pharmaceuticals, College of Life and Health Sciences, Northeastern University.

Extraction and Isolation Dried rhizomes of *S. ningpoensis* (19 kg) were extracted with 70% EtOH (50 L $\times$ 3) under reflux, and the solvent was evaporated *in vacuo*. This concentrate was dissolved in water and partitioned with ethyl acetate to obtain an ethyl acetate extract (200 g). The dried EtOAc extract was subjected to silica gel column chromatography, eluting with a gradient of CH_2Cl_2 –MeOH to afford 8 fractions (fractions 1–8). Fraction 4 (7 g) was subjected to Sephadex LH-20 chromatography (MeOH) to produce 12 subfractions (fractions 4.1–4.12). Fraction 4.5 (970 mg) was separated by silica gel column chromatography, eluting with CH_2Cl_2 –MeOH=30:1 to afford **8** (20.0 mg) and the residues was subjected to ODS column chromatography, eluting with methanol–water (40:60) to yield **7** (18.0 mg). Fraction 4.6 (1.5 g) was separated by silica gel column chromatography, eluting with CH_2Cl_2 –MeOH=30:1 to produce 16 subfractions (fractions 4.6.1–4.6.16). Subfraction 4.6.13 (190 mg) was subjected to silica gel column chromatography, eluting with CH_2Cl_2 –acetone=3:1 to afford a mixture of **3a** and **b** (7.0 mg). Subfraction 4.6.16 (65 mg) was subjected to silica gel column chromatography, eluting with CH_2Cl_2 –MeOH=30:1 to afford a mixture of **5a** and **b** (16.0 mg). Fraction 4.7 (198 mg) was separated by silica gel column chromatography, eluting with CH_2Cl_2 –acetone=7:1 to yield 8 subfractions (fractions 4.7.1–4.7.8). Subfraction 4.7.2 (30 mg) was subjected to ODS column chromatography, eluting with methanol–water (45:55) to yield **6** (2.0 mg). Fraction 5 (8 g) was separated using Sephadex LH-20 chromatography (MeOH) to produce 7 subfractions (fractions 5.1–5.7). Fraction 5.4 (2 g) was subjected to silica gel column chromatography (CH_2Cl_2 –MeOH=20:1) to produce 9 subfractions (fractions 5.4.1–5.4.9). Subfraction 5.4.1 (200 mg) was subjected to silica gel column chromatography (CH_2Cl_2 –acetone=10:1) to yield **1** (2.7 mg). Subfraction 5.4.8 (60 mg) was subjected to silica gel column chromatography (CH_2Cl_2 –acetone=2:1) to yield **9** (3.3 mg). Fraction 6 (7 g) was separated using Sephadex LH-20 chromatography (MeOH) to produce 7 subfractions (fractions 6.1–6.7). Fraction 6.2 (970 mg) was subjected to silica gel column chromatography (CH_2Cl_2 –MeOH=25:1) to produce 9 subfractions (fractions 6.2.1–6.2.9). Subfraction 6.2.3 (18 mg) was subjected to silica gel column chromatography (CH_2Cl_2 –acetone=3:1) to yield **2** (1.5 mg). Subfraction 6.2.8 (30 mg) was subjected to silica gel column chromatography (CH_2Cl_2 –acetone=3:1) to yield **4** (3.3 mg). Fraction 6.3 (930 mg) was subjected to silica gel column chromatography (CH_2Cl_2 –MeOH=25:1) to produce 10 subfractions (fractions 6.3.1–6.3.10). Subfraction 6.3.7 (64 mg) was subjected to silica gel column chromatography (CH_2Cl_2 –MeOH=12:1) to yield **10** (5.0 mg). Fraction 6.6 (100 mg) was subjected to silica gel column chromatography (CH_2Cl_2 –MeOH=30:1) to yield **11** (2.0 mg).

1 β ,6 β -Dimethoxy-dihydrocatalpolgenin (1)

Colorless oil; $[\alpha]_{\text{D}}^{20} -80.0$ ($c=0.4$, MeOH); IR (film) cm^{-1} : 3457, 2934, 2852, 1393, 1202, 1120, 1086, 1057, 991, 968. $^1\text{H-NMR}$ (600 MHz, dimethyl sulfoxide (DMSO)- d_6) and $^{13}\text{C-NMR}$ (150 MHz, $\text{DMSO}-d_6$) data, see Table 1; ESI-MS

m/z 253 $[M+Na]^+$; HR-ESI-MS m/z 253.1045 $[M+Na]^+$ (Calcd for $C_{11}H_{18}O_5Na$, 253.1052).

Ningpogeniridoid (2)

Colorless oil; $[\alpha]_D^{20}$ -80.0 ($c=0.3$, MeOH); IR (film) cm^{-1} : 3385, 2927, 2855, 1643, 1453, 1238, 1179, 1111, 1090, 1038, 974, 955. 1H -NMR (600MHz, DMSO- d_6) and ^{13}C -NMR (150MHz, DMSO- d_6) data, see Table 1; ESI-MS m/z 253 $[M+Na]^+$; HR-ESI-MS m/z 253.1054 $[M+Na]^+$ (Calcd for $C_{11}H_{18}O_5Na$, 253.1052).

1 β -Hydroxy-6 β -methoxy-dihydrocatalpolgenin (3a) and 1 α -Hydroxy-6 β -methoxy-dihydrocatalpolgenin (3b)

Colorless oil; $[\alpha]_D^{20}$ -45.5 ($c=1.0$, MeOH); IR (film) cm^{-1} : 3385, 2936, 1648, 1107, 1083, 1057, 1017, 994. 1H -NMR (600MHz, DMSO- d_6) and ^{13}C -NMR (150MHz, DMSO- d_6) data, see Table 1; ESI-MS m/z 239 $[M+Na]^+$; HR-ESI-MS m/z 239.0891 $[M+Na]^+$ (Calcd for $C_{10}H_{16}O_5Na$, 239.0895).

1-Ethoxyl-3-hydroxy-2,3-seconingpogenin (4)

Colorless oil; $[\alpha]_D^{20}$ $+62.9$ ($c=0.7$, MeOH); IR (film) cm^{-1} : 3373, 3333, 2959, 2926, 1650, 1443, 1331, 1160, 1123, 1061, 992. 1H -NMR (600MHz, DMSO- d_6) and ^{13}C -NMR (150MHz, DMSO- d_6) data, see Table 2; ESI-MS m/z 239 $[M+Na]^+$; HR-ESI-MS m/z 239.1259 $[M+Na]^+$ (Calcd for $C_{11}H_{20}O_4Na$, 239.1259).

Ninpopyrrosides A and B (5a/b)

Colorless oil; $[\alpha]_D^{20}$ $+7.7$ ($c=1.04$, MeOH); IR (film) cm^{-1} : 3393, 3275, 2949, 2927, 2872, 1692, 1453, 1422, 1333, 1280, 1058, 958. 1H -NMR (600MHz, DMSO- d_6) and ^{13}C -NMR (150MHz, DMSO- d_6) data, see Table 2; ESI-MS m/z 254 $[M+H]^+$, 276 $[M+Na]^+$; HR-ESI-MS m/z 254.1390 $[M+H]^+$ (Calcd for $C_{13}H_{20}NO_4$, 254.1392).

Inhibition of NO Production Assay The anti-inflammatory activity of the isolated compounds **1–11** were evaluated through inhibition of NO production in lipopolysaccharide-induced RAW264.7 macrophage cells using the Griess method²⁵ and experiments were carried out as our previous descriptions.²⁶ Minocycline was used as a positive control.

Cytotoxicity Assay The cytotoxicities of **1–11** were assayed against three human carcinoma cell lines including a human gastric carcinoma cell line (SGC-7901), a human lung carcinoma cell line (H1975), and a human hepatocellular carcinoma cell line (SMMC-7721) in a 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay.²⁶ Etoposide (VP-16) was assayed as a positive control.

Antimicrobial Assay A micro broth dilution assay²⁷ was used to evaluate antimicrobial activity of **1–11** against *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC MYA-2876, and *Candida parapsilosis* ATCC 22019. The minimum inhibitory concentrations (MICs) were defined as the lowest concentration of the antimicrobial agent that completely inhibited the visual growth of an organism. Ciprofloxacin and amphotericin B were used as positive controls against bacteria and fungi, respectively.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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