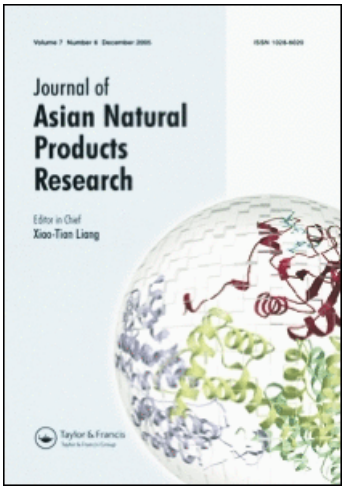

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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Yan Lou[a]; Feng Zhao[b]; Hao He[a]; Kai-Feng Peng[a]; Xiao-Hua Zhou[a]; Li-Xia Chen[a]; Feng Qiu[a]
[a] Department of Natural Products Chemistry, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China [b] School of Pharmacy, Yantai University, Yantai, China

To cite this Article Lou, Yan , Zhao, Feng , He, Hao , Peng, Kai-Feng , Zhou, Xiao-Hua , Chen, Li-Xia and Qiu, Feng(2009) 'Guaiane-type sesquiterpenes from *Curcuma wenyujin* and their inhibitory effects on nitric oxide production', Journal of Asian Natural Products Research, 11: 8, 737 — 747
To link to this Article: DOI: 10.1080/10286020903042358
URL: http://dx.doi.org/10.1080/10286020903042358

Journal of Asian Natural Products Research
Vol. 11, No. 8, August 2009, 737–747

Guaiane-type sesquiterpenes from *Curcuma wenyujin* and their inhibitory effects on nitric oxide production

Yan Lou[a], Feng Zhao[b], Hao He[a], Kai-Feng Peng[a], Xiao-Hua Zhou[a], Li-Xia Chen[a] and Feng Qiu[a]*

[a]*Department of Natural Products Chemistry, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China;* [b]*School of Pharmacy, Yantai University, Yantai 264005, China*

(*Received 24 March 2009; final version received 13 May 2009*)

Four new guaiane-type sesquiterpenes (1*S*,4*S*,5*S*,10*R*)-zedoarondiol, zedoalactones D, E, and F (**1**–**4**), along with 10 known ones (**5**–**14**), were isolated from *Curcuma wenyujin* Y.H. Chen et C. Ling. The structures of these new compounds were elucidated by spectroscopic methods. The inhibitory effects of compounds **1**–**14** on nitric oxide production in lipopolysaccharide-activated macrophages were evaluated.

Keywords: *Curcuma wenyujin*; guaiane; sesquiterpenes; nitric oxide; zedoalactone D, E, F

1. Introduction

The *Curcuma* drugs have multiple pharmacological effects such as stomachic [1], carminative [1], anti-inflammatory [2], anti-tumor [3], and immunological activities [4]. In traditional Chinese and Japanese medicine, *Curcuma* drugs are generally used to treat the Oketsu syndromes (various syndromes caused by the obstruction of blood circulation such as arthralgia, psychataxia, and dysmenorrhea) [5]. The essential oil of *Curcuma wenyujin* is currently embodied in the pharmacopoeia of the P.R. China (2005), as an anti-cancer and anti-virus remedy [6]. Previous chemical investigations on the genus *Curcuma* have led to the isolation of sesquiterpenoids [1,7,8] and diarylheptanoids [9,10], and some of these compounds possess significant vasorelaxant [1,11] and hepatoprotective activities [12,13]. Our investigation of bioactive constituents from the dried rhizomes of *C. wenyujin* has resulted in the isolation of four new guaiane-type sesquiterpenes (**1**–**4**), together with 10 known compounds (**5**–**14**) (Figure 1). We describe herein the isolation and characterization of these compounds and their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated macrophages.

2. Results and discussion

The dried rhizomes of *C. wenyujin* were extracted successively with cyclohexane and 50% ethanol. The 50% ethanol extract was partitioned successively with cyclohexane, EtOAc, and *n*-BuOH to yield three fractions. These fractions were purified by repeated column chromatography, including Silica gel, Sephadex LH-20 column, ODS column, HPLC, and preparative-TLC

*Corresponding author. Email: fengqiu2000@tom.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020903042358
http://www.informaworld.com

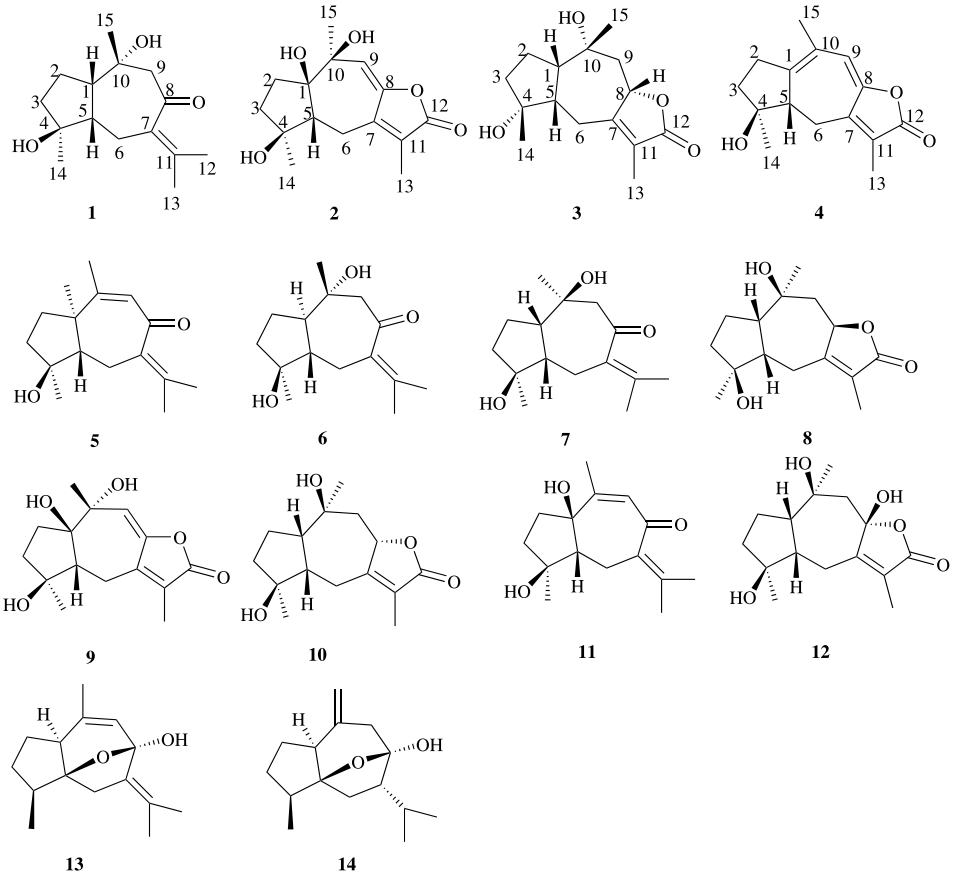

Figure 1. Chemical structures of compounds **1**–**14**.

(P-TLC) to afford four new compounds and 10 known compounds.

Compound **1** was obtained as a colorless oil with the molecular formula $C_{15}H_{24}O_3$ as deduced by HR-ESI-MS. The IR spectral data showed a hydroxyl group (3361 cm^{-1}) and a carbonyl group (1669 cm^{-1}), and the ^{13}C NMR spectrum exhibited 15 carbon signals composed of four methyls (δ_C 22.9, 22.2, 24.7, and 29.0), four methylenes (δ_C 24.1, 37.4, 28.3, and 52.9), two methines (δ_C 52.7 and 54.1), two quaternary oxygenated carbons (δ_C 83.2 and 72.9), two olefinic carbons (δ_C 135.3 and 145.2), and a ketone carbonyl carbon (δ_C 205.5). These functionalities accounted for two out of the four degrees of unsaturation, and the remaining two degrees of unsaturation required compound **1** to be bicyclic. The aforementioned spectroscopic features implied that compound **1** was a guaiane-type sesquiterpene [14–16]. The HMBC correlations (Figure 3) of H-14 with C-3, C-4, C-5; H-15 with C-1, C-9, C-10; H-13 with C-7, C-8, C-11, C-12, together with H-1 with C-2, C-5, C-10; and H-6 with C-1, C-4, C-5, C-7, C-8, C-11 completed the planar structure of **1**. The relative configuration of **1** was established from the NOESY spectrum (Figure 2). In the NOESY spectrum, the correlations of H-1/H-5, H-1/H-15, and H-5/H-15 indicated that they were in the same orientation. The correlations of H-14/H-6α and H-14/H-6β but no correlation between

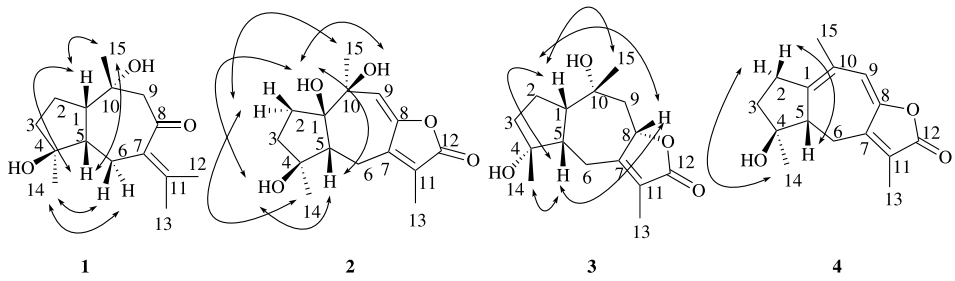

Figure 2. The key NOESY correlations of compounds **1**–**4**.

H-14 and H-5 suggested that the H-14 and H-5 were in an opposite orientation. The hypothesis was supported by a biosynthetic aspect [17] and molecular modeling. Comparing the molecular structure of **1** with that of the known compound zedoarondiol (**6**) [18], **1** has the same planar structure as **6**, whose absolute stereostructure was established by the circular dichroism (CD) and the X-ray analysis [18]. The absolute stereochemistry of **1** was determined by the CD spectrum. The CD curve of **1** showed a negative cotton effect (327 nm, MeOH) that attributed to the $n-\pi^*$ transition of an α,β-unsaturated ketone [19–21] and a negative cotton effect (254 nm, MeOH) that attributed to the $\pi-\pi^*$ transition of an α,β-unsaturated ketone [19–21]. The NOESY spectrum of **1** showed the correlation between H-15 and H-9α, which had a parallel relationship in the molecular modeling. The molecular modeling along with the CD spectrum indicated that the configuration of **1** had anticlockwise helicity of the conjugated enone system. Thus, **1** was assigned as (1*S*,4*S*,5*S*,10*R*)-zedoarondiol.

Niu jia *et al.* [22] reported a guaiane-type sesquiterpene, and it was assigned as (1*S*,4*S*,5*S*,10*R*)-isozedoarondiol. The ^{1}H NMR, ^{13}C NMR, and IR data of **1** were similar to those of (1*S*,4*S*,5*S*,10*R*)-isozedoarondiol, but the optical properties, such as the optical rotation and the CD spectrum, were opposite. Therefore, **1** is a new compound.

Compound **2**, obtained as a colorless gum, had the molecular formula $C_{15}H_{20}O_5$ as determined by HR-ESI-MS. Its IR spectrum showed absorption bands for a hydroxyl group (3440 cm^{-1}) and an α,β-unsaturated γ-lactone ring (1670, 1740, 1121 cm^{-1}). The UV spectrum revealed an absorption maximum at 275 nm. The NMR data (C_5D_5N) (Tables 1 and 2) indicated the presence of an ester carbonyl carbon (δ_C 171.4), three methyl carbons (δ_C 8.5, 23.7, and 28.3), three methylene carbons (δ_C 35.4, 41.0, and 24.6), one methine carbon (δ_C 63.3), three quaternary oxygenated carbons (δ_C 87.7, 80.8, and 74.3), and four olefinic carbons (δ_C 152.1, 152.8, 121.2, and 124.2). Analysis of these data of **2** indicated that it had a guaiane sesquiterpene carbon skeleton identical to that of zedoalactone B (**9**) [16]. The similarity of the mass, IR, UV, and ^{1}H and ^{13}C NMR spectra of **2** and **9** indicated that **2** was a diastereomer of **9**, and the HMBC correlations (Figure 3) supported the deduction. Careful comparison of the ^{13}C NMR data of **2** with those of **9** revealed that the C-10 signal of **2** shifted upfield to δ_C 74.3 from δ_C 82.7, and C-1 and C-5 signals of **2** downfield to δ_C 87.7 from δ_C 75.1 and to δ_C 63.3 from δ_C 50.3, respectively. On the basis of these findings, the C-1, C-5, and C-10 in **2** may have their orientations changed. This assignment was confirmed by the NOESY spectrum (Figure 2). The correlations of OH-1/H-5, H-5/OH-4,

Table 1. ^{1}H NMR spectral data (δ) for compounds **1**–**4** (δ in ppm, J in Hz).

Position	**1**[a,b]	**1**[c,d]	**2**[a,d]	**2**[d,e]	**3**[a,d]	**4**[a,b]
1	2.86 m	2.71 m			2.00 m	
2	1.91 m, 1.91 m	1.69 m, 1.69 m	1.59 m (β), 2.08 m (α)	2.09 m (β), 2.61 m (α)	1.64 m, 1.83 m	2.32 m (α), 2.51 m (β)
3	1.75 m, 1.75 m	1.57 m, 1.57 m	2.08 m (α), 1.67 m (β)	2.59 m (α), 2.04 m (β)	1.62 m (α), 1.69 m (β)	1.89 m (α), 1.76 brd (15.9, β)
5	1.80 m	1.62 m	2.00 dd (13.2, 3.8)	2.88 dd (13.2, 3.6)	1.56 ddd (2.5, 9.2, 12,4)	2.55 m
6	2.50 brd (13.8), 1.94 m	2.30 brd (13.8), 1.80 m	3.15 t (14.2, β), 2.78 dd (14.2, 3.8, α)	3.69 t (13.2, β), 3.14 dd (13.2, 3.6, α)	3.03 dd (15.0, 2.5, β), 2.16 dd (15.0, 12.4, α)	2.89 dd (15.6, 1.8, α), 2.19 ddd (1.8, 2.1, 15.6, β)
8					5.18 brd (10.9)	
9	3.38 d (15.7), 2.24 d (15.7)	3.24 d (15.6), 2.07 d (15.6)	5.75 s	6.26 s	1.73 m (α), 2.24 dd (2.8, 14.6, β)	5.94 s
12	1.93 s	1.84 s				
13	1.87 s	1.78 s	1.89 s	1.70 s	1.79 s	1.94 s
14	1.36 s	1.24 s	1.26 s	1.74 s	1.22 s	1.30 s
15	1.22 s	1.08 s	1.42 s	1.86 s	1.18 s	1.85 s
1-OH		4.30 s		6.17 s		
4-OH		4.30 s		6.40 s		
10-OH		3.50 s		7.10 s		

[a] Recorded in MeOH-d_4.
[b] Obtained at 300 MHz.
[c] Recorded in DMSO.
[d] Obtained at 600 MHz.
[e] Recorded in C_5D_5N.

Table 2. ^{13}C NMR spectral data (δ) for compounds **1**–**4** (δ in ppm, J in Hz).

Position	**1**[a,b]	**1**[c,d]	**2**[a,b]	**2**[d,e]	**3**[a,b]	**4**[a,b]
1	52.7	51.2	88.1	87.7	54.1	144.7
2	24.1	23.2	34.7	35.4	24.3	30.8
3	37.4	36.6	40.0	41.0	41.5	40.0
4	83.2	80.6	81.0	80.8	80.9	81.1
5	54.1	52.6	62.4	63.3	49.5	52.1
6	28.3	26.7	24.2	24.6	30.2	25.1
7	135.3	134.4	152.2	152.1	166.2	148.4
8	205.5	202.9	153.4	152.8	81.1	150.9
9	52.9	52.3	120.1	121.2	47.0	115.0
10	72.9	70.8	74.3	74.3	73.1	123.5
11	145.2	140.8	125.0	124.2	122.4	123.6
12	22.9	22.1	173.0	171.4	176.7	170.9
13	22.2	21.4	8.2	8.5	8.4	8.9
14	24.7	24.8	22.4	23.7	23.3	23.1
15	29.0	29.0	27.3	28.3	23.0	21.2

[a] Recorded in CD_3OD.
[b] Obtained at 75 MHz.
[c] Recorded in DMSO.
[d] Obtained at 150 MHz.
[e] Recorded in C_5D_5N.

OH-1/OH-4, and OH-1/OH-10 indicated that they were in the same orientation, while the correlation between H-2α/H-14 and H-2α/H-15 suggested that H-15 and H-14 were in the α-orientation. Thus, the structure of **2** was determined to be 1β,4β,10β-trihydroxy-5β*H*-guai-7(11),8-dien-12,8-olide, namely, zedoalactone D.

Compound **3** was formulated as $C_{15}H_{22}O_4$ with five degrees of unsaturation by HR-ESI-MS. The UV spectrum (218 nm) and the IR spectrum (3420, 1734, 1667, 1117 cm^{-1}) suggested the presence of an α,β-unsaturated γ-lactone ring. This was supported by the degrees of unsaturation of **3**. The ^{1}H NMR and ^{13}C NMR spectra were similar to those of **2** (CD_3OD), except for the emergence of methylene (δ_H 2.24, δ_H 1.73, δ_C 47.0) and two additional methines (δ_H 2.00, δ_C 54.1 and δ_H 5.18, δ_C 81.1), and the disappearance of the oxygenated quaternary carbon (δ_C 88.1) and a $\Delta^{8,9}$ double bond (δ_C 153.4, 120.1). The HMBC correlations (Figure 3) of H-1 with C-3, C-5, C-10, and C-15; and H-9 with C-8, C-10, and C-15 further confirmed that C-1 had changed from an oxygenated quaternary carbon to the methylene and C-8, C-9 had changed from a double bond to two methines. On the basis of the above analysis, the planar structure of **3** was elucidated.

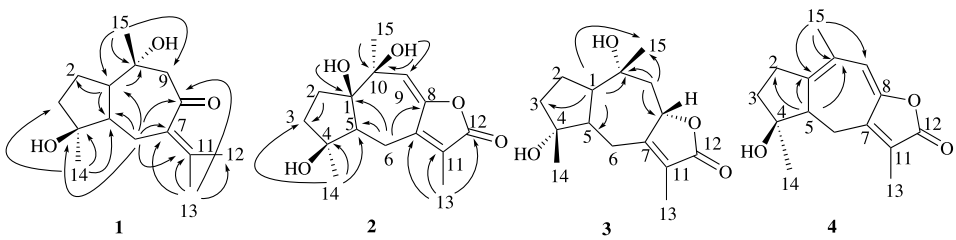

Figure 3. The key HMBC correlations of compounds **1**–**4**.

The relative stereochemistry of **3** was determined by NOE correlations (Figure 2) and $^{1}H-^{1}H$ coupling constants. The value of $J_{H\text{-}1}/J_{H\text{-}5}$ (9.2 Hz) and a NOE correlation of H-1/H-5 indicated that the H-1 and H-5 had a *cis*-configuration. Furthermore, the correlations between H-8 and H-1; H-8 and H-5; H-1 and H-15; and H-5 and H-14 suggested all to be in the same β-orientation. Thus, compound **3** was determined to be 4α,10α-dihydroxy-1,5,8β*H*-guai-7(11)-en-12,8-olide, namely, zedoalactone E.

Compound **4** possessed the elemental composition $C_{15}H_{18}O_3$ as determined by HR-ESI-MS and NMR analyses. The IR absorption implied the presence of α,β-unsaturated γ-lactone (1750 cm^{-1}) and hydroxyl (3450 cm^{-1}) functionalities. The UV spectrum (229, 272.6, 356 nm) of **4** showed a marked bathochromic shift compared with that of compound **3**, indicating the presence of another unsaturated bond conjugated with the lactone ring. Its ^{1}H NMR and ^{13}C NMR were nearly similar to those of **2** (CD_3OD), except for the appearance of the two additional olefinic carbons (δ_C 144.7, C-1, δ_C 123.5, C-10), and the disappearance of two oxygenated quaternary carbons (δ_C 88.1, C-1, δ_C 74.3, C-10). The HMBC (Figure 3) correlations of H-15 with C-1, C-9, and C-10; and H-5 with C-1, C-2, and C-10 incorporated the $\Delta^{1,(10)}$ double bond between C-5 and C-15. On the basis of the aforementioned information, the planar structure of **4** was deduced. The relative stereochemistry of **4** was established from the NOESY spectrum (Figure 2). The NOE correlations between Me-14 (δ_H 1.30), H-3α (δ_H 1.89), and H-2α (δ_H 2.32) indicated that they were cofacial and were randomly assigned as α-orientation. The correlation of H-5 (δ_H 2.55) and H-2β (δ_H 2.51) indicated that H-5 was in the β-orientation. Thus, the structure of compound **4** was determined to be 4β-hydroxy-5β*H*-guai-1(10),7(11), 8-trien-12,8-olide, namely, zedoalactone F.

In addition to four new sesquiterpenes (**1**–**4**), 10 known compounds, procurcumenol [23] (**5**), zedoarondiol [18] (**6**), isozedoarondiol [18] (**7**), zedoalactone A [16] (**8**), zedoalactone B [16] (**9**), zedoalactone C [14] (**10**), aerugidiol [17] (**11**), zedoarolide B [24] (**12**), curcumenol [25] (**13**), and curcumol [26] (**14**), were also isolated and identified by comparison of their spectroscopic data with those reported in the literature. The chemical structures of compounds **1**–**14** are shown in Figure 1.

Compounds **1**–**14** were examined for their inhibitory effects on NO production induced by LPS in macrophages (Table 3). Cell viability in the present experiment was determined by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) method to find whether inhibition of NO production was due to cytotoxicity of test compounds (data not shown), and curcumol showed moderate cytotoxicity. As shown in Table 3, hydrocortisone (IC_{50} 53.78 ± 4.84 μM) was used as a positive control. Compounds **9** and **14** showed strong inhibition of NO production induced by LPS. Compound **13** exhibited moderate activities, which were close to that of hydrocortisone. Compound **4** showed very weak activities.

Since NO is the relaxation factor of vascular smooth muscle and also an inhibitor of platelet aggregation in blood vessels [27], the inhibitory activity of these compounds against NO production may be an important evidence substantiating the traditional effects of this herbal medicine for the treatment of 'Oketsu' syndrome caused by blood stagnation with inflammation.

3. Experimental

3.1 General experimental procedures

UV spectra were recorded with a Shimadzu UV2201 spectrophotometer in MeOH, and NMR experiments were performed on Bruker ARX-300 and 600

Table 3. Inhibitory effects of compounds **1**–**14** on NO production induced by LPS in RAW 264.7 cells[a].

	Inhibitory rate on the NO production				
	Concentration (μM)				
Compound	100	30	10	3	IC_{50} (μM)
1	34.4 ± 1.2	28.7 ± 2.6	18.8 ± 1.9	12.3 ± 1.9	>100
2	32.2 ± 2.0	19.9 ± 3.2	9.8 ± 2.4	4.7 ± 1.0	>100
3	10.4 ± 2.8	5.8 ± 1.5	6.2 ± 3.1	1.9 ± 0.7	>100
4	54.2 ± 4.0	33.4 ± 4.6	28.2 ± 1.5	10.9 ± 2.4	92.6
5	46.6 ± 4.2	38.1 ± 3.6	30.2 ± 2.9	23.6 ± 2.4	>100
6	17.0 ± 3.9	15.3 ± 3.4	18.2 ± 0.8	3.4 ± 2.0	>100
7	−4.3 ± 0.6	0.2 ± 0.4	1.1 ± 0.6	−2.3 ± 0.9	>100
8	11.4 ± 3.9	8.6 ± 2.1	3.2 ± 0.8	3.8 ± 1.3	>100
9	81.7 ± 5.0	57.7 ± 1.9	33.0 ± 2.3	31.6 ± 2.0	23.8
10	3.7 ± 3.5	2.8 ± 1.7	3.2 ± 0.8	1.5 ± 0.6	>100
11	15.3 ± 1.0	12.4 ± 2.3	6.5 ± 1.2	2.8 ± 1.1	>100
12	−7.9 ± 4.8	−3.4 ± 1.2	0.5 ± 2.0	1.8 ± 1.2	>100
13	69.9 ± 5.2	43.7 ± 2.4	36.7 ± 3.5	27.5 ± 1.8	46.8
14	101.8 ± 1.1[b]	73.5 ± 2.9	42.4 ± 4.4	2.9 ± 0.6	14.9
Hydrocortisone	88.7 ± 4.4	32.9 ± 2.7	25.4 ± 3.6	18.7 ± 3.7	53.8

[a] NO concentration of the control group: 2.6 ± 0.6 μM; NO concentration of the LPS-treated group: 27.6 ± 1.2 μM.
[b] Cytotoxicity.

spectrometers using TMS as an internal standard. ESI-MS data were measured on an Agilent 1100-LC/MSDTrapSL mass spectrometer. IR spectra were measured with a Bruker IFS 55 spectrometer. HR-ESI-MS data were measured on a Bruker APEX II mass spectrometer. CD spectra were recorded on a Chirascan spectrometer (Applied Photophysics Co., Leatherhead, UK). Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Silica gel GF 254 prepared for TLC and silica gel (200–300 mesh) for column chromatography were obtained from Qingdao Marine Chemical Company (Qingdao, China). Sephadex LH-20 is a product of Amersham Co. RP-18 (10–75 μm) silica gel was purchased from Merck Chemical Ltd. Preparative HPLC was carried out on a Waters 600 apparatus with an ODS column (C18, 250 × 20 mm; Inertsil Pak). All reagents were purchased from Shenyang Chemical Company (Shenyang, China).

3.2 Bioassay for NO production

Mouse monocyte–macrophage RAW 264.7 cells (ATCC TIB-71) were purchased from the Chinese Academy of Sciences. RPMI 1640 medium, penicillin, streptomycin, and fetal bovine serum were purchased from Invitrogen (New York, NY, USA). LPS, dimethylsufoxide (DMSO), MTT, and hydrocortisone were obtained from Sigma Co. RAW 264.7 cells were suspended in RPMI 1640 medium supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% heat-inactivated fetal bovine serum. The cells were harvested with trypsin and diluted to a suspension in fresh medium. The cells were seeded in 96-well plates with 1×10^5 cells/well and allowed to adhere for 2 h at 37°C in 5% CO_2 in air. Then, the cells were treated with 1 μg/ml of LPS for 24 h with or without various concentrations of test compounds. DMSO was used as a solvent for the test compounds, which were applied at a final concentration

of 0.2% (v/v) in cell culture supernatants. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using Griess reagent [28]. Briefly, 100 μl of the supernatant from incubates was mixed with an equal volume of Griess reagent (0.1% *N*-[1-naphthyl]ethylenediamine and 1% sulfanilamide in 5% H_3PO_4). Cytotoxicity was determined by the MTT colorimetric assay, after 24 h incubation with test compounds. The concentration of NO_2^- was calculated by a working line from 0, 3, 10, 30, and 100 μM sodium nitrite solutions, and the inhibitory rate on NO production induced by LPS was calculated by the NO_2^- levels as follows:

$$\text{Inhibitory rate (\%)} = 100 \times \frac{[NO_2^-]_{LPS} - [NO_2^-]_{LPS+sample}}{[NO_2^-]_{LPS} - [NO_2^-]_{untreated}}.$$

Experiments were performed in triplicate, and data are expressed as the mean ± SD of three independent experiments.

3.3 Plant material

The dried rhizomes of *C. wenyujin* (9.0 kg) were collected from Wenzhou, China, in 2004. A voucher specimen (No. 20040919) was identified by Prof. Qi-shi Sun and has been deposited at the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, China.

3.4 Extraction and isolation

The dried rhizomes of *C. wenyujin* (9.0 kg) were extracted with cyclohexane and filtered. The residue was then extracted with 50% EtOH. The alcoholic filtrate was concentrated, and suspended in water, which was partitioned successively with cyclohexane, EtOAc, and *n*-BuOH. The combined cyclohexane extract (55.0 g) was chromatographed on a silica gel (200–300 mesh 500 g, 10 × 100 cm) column, using gradient elution with cyclohexane–acetone (100:1–0:100) to yield fractions 1–12. Fraction 5 (2.0 g) was subjected to Sephadex LH-20 with $CHCl_3$–MeOH (1:1) to give four major sub-fractions (51–54). Fraction 52 (740.0 mg) was separated on an ODS column with MeOH–H_2O (1:9–10:0), and then purified by P-HPLC using MeOH–H_2O (45:55) to yield compound **13** (33.0 mg) and compound **14** (230.5 mg). The EtOAc fraction (78.0 g) was chromatographed on silica gel (200–300 mesh, 550 g, 10 × 100 cm), using a gradient of $CHCl_3$–MeOH (100:1–0:100), which yielded 19 fractions (1–19). Fraction 3 (3.8 g) was subjected to silica gel using $CHCl_3$–MeOH (100:1–0:100) to afford 10 sub-fractions (31–310). Fractions 3–9 (267.0 mg) were subjected to ODS using a gradient solvent system of MeOH–H_2O (0:100–100:0) followed by HPLC using MeOH–H_2O (55:45) to yield compound **5** (104.6 mg). Fraction 13 (2.5 g) was separated by Sephadex LH-20 ($CHCl_3$–MeOH 1:1) to give four sub-fractions (131–134). Fraction 132 (350.0 mg) was applied to an ODS column using a gradient solvent system of MeOH–H_2O (0:100–100:0), and then purified by HPLC using MeOH–H_2O (42:58) to yield compound **2** (4.5 mg) and compound **6** (52.3 mg). Fraction 133 (76.0 mg) was purified by P-TLC ($CHCl_3$–MeOH 10:1) to obtain compound **7** (17.2 mg). Fraction 14 (5.8 g) was chromatographed over silica gel using a gradient of $CHCl_3$–MeOH (100:1–0:100), which gave eight sub-fractions (141–148). Fraction 143 (1.2 g) was applied to an ODS column using a gradient solvent system of MeOH–H_2O (0:100–100:0) and followed by HPLC using MeOH–H_2O (32:68) to obtain compound **4** (5.5 mg), compound **8** (17.5 mg), compound **9** (13.9 mg), compound **10** (21.0 mg), and compound **12** (10.2 mg), respectively. Fraction 15 (1.9 g) was subjected to ODS using a gradient solvent system of MeOH–H_2O (0:100–100:0) followed by HPLC using MeOH–H_2O (38:62) to yield compound **11**

(50.6 mg). Fraction 16 (1.2 g) was chromatographed on Sephadex LH-20 ($CHCl_3$–MeOH 1:1) to yield five sub-fractions (161–165). Fraction 162 (234.0 mg) was separated by the ODS column eluted with MeOH–H_2O (3:7–10:0) to afford fraction 1623 (132.0 mg), which was further purified by HPLC using MeOH–H_2O (32:68) to give compound **1** (15.1 mg). The *n*-BuOH fraction (25.0 g) was chromatographed on DA-201 eluted with a gradient of EtOH–H_2O (10:90–0:100) to yield five fractions (1–5). Fraction 4 (3.4 g) was applied to the Sephadex LH-20 column eluted with MeOH–H_2O (1:1) to yield compound **3** (18.1 mg).

3.4.1 (1S,4S,5S,10R)-Zedoarondiol (*1*)

Colorless oil; $[\alpha]_D^{25}$ −32.0 ($c = 0.1$, MeOH); CD ($c = 5.5 \times 10^{-3}$, MeOH) $[\theta]_{322} = -550$, $[\theta]_{254} = -1700$; UV (MeOH) λ_{max} (log ε): 249 (3.7), 201 (3.7) nm; IR (KBr) ν_{max}: 3361 (OH), 2935, 1669, 1375, 1151 cm^{-1}; ^1H NMR (Table 1); ^{13}C NMR (Table 2); HR-ESI-MS *m/z*: 275.1619 $[M+Na]^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1618).

3.4.2 Zedoalactone D (*2*)

Colorless gum; $[\alpha]_D^{25}$ −52.5 ($c = 0.08$, MeOH); UV (MeOH) λ_{max} (log ε): 275 (3.86) nm; IR (KBr) ν_{max}: 3440 (OH), 1740, 1670, 1121 cm^{-1} (α,β-unsaturated γ-lactone); ^1H NMR (Table 1); ^{13}C NMR (Table 2); HR-ESI-MS *m/z*: 303.1216 $[M+Na]^+$ (calcd for $C_{15}H_{20}O_5Na$, 303.1208).

3.4.3 Zedoalactone E (*3*)

Colorless oil; $[\alpha]_D^{25}$ +11.5 ($c = 0.375$, MeOH); UV (MeOH) λ_{max} (log ε): 218 (4.03) nm; IR (KBr) ν_{max}: 3420 (OH), 1734, 1667, 1117 cm^{-1} (α,β-unsaturated γ-lactone); ^1H NMR (Table 1); ^{13}C NMR (Table 2); HR-ESI-MS *m/z*: 289.1411 $[M+Na]^+$ (calcd for $C_{15}H_{22}O_4Na$, 289.1416).

3.4.4 Zedoalactone F (*4*)

Colorless oil; $[\alpha]_D^{25}$ −55.0 ($c = 0.1$, MeOH); UV (MeOH) λ_{max} (log ε): 229 (3.34), 272.6 (3.28), 356.0 (3.34) nm; IR (KBr) ν_{max}: 3450 (OH), 1750, 1680, 1131 cm^{-1} (α,β-unsaturated γ-lactone); ^1H NMR (Table 1); ^{13}C NMR (Table 2); HR-ESI-MS *m/z*: 269.1144 $[M+Na]^+$ (calcd for $C_{15}H_{18}O_3Na$, 269.1154).

Acknowledgements

The authors are grateful to Liaoning Natural Science Foundation for financial support (No. 20062057).

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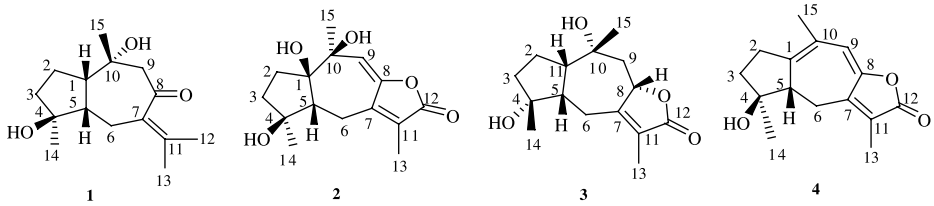

1 **2** **3** **4**

Four new guaiane-type sesquiterpenes (1*S*,4*S*,5*S*,10*R*)-zedoarondiol, zedoalactone D, E, and F (**1**–**4**) were isolated from *Curcuma wenyujin* Y.H. Chen et C. Ling. Their inhibitory effects on nitric oxide production in lipopolysaccharide-activated macrophages were evaluated.

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