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Two Anti-inflammatory Diterpenes from the Rhizomes of *Alpinia Officinarum* Hance

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ABSTRACT

In this study, two labdane diterpenes, (12*S*)-15,16-epoxy-8(17),13(16),14-labdatrien-12-ol(**1**) and (12*E*)-labda-8(17),12(13)-dien-15,16-olide(**2**), were isolated from the rhizomes of *Alpinia officinarum*. They were identified by comparing the spectroscopic data with those in the literatures. Both of them were isolated from this plant for the first time. Besides, they were found to own strong anti-inflammatory and antioxidant activities.

KEYWORDS *Alpinia officinarum*, diterpenes, anti-inflammatory, antioxidant

INTRODUCTION

Alpinia officinarum Hance, also named "lesser galangal", is a traditional medicinal herb in China. Its rhizomes have been contributed to the formulation of some Chinese medicines that are widely used for the relief of symptoms such as stomach aches, colds, ulcer and diarrhea[1]. Health products containing constituents of *Alpinia officinarum* are available in the market like health beverage or spices. Over the years, considerable studies have disclosed and testified that flavonoids, volatile oil and diarylheptanoids have made up the main compositions of *Alpinia officinarum*[2-6], while the reports on the diterpenes were very rare. It has been reported to have anti-inflammatory[7], anti-oxidant[8], antiviral[9], antiemetic[10], anti-cancer[11] and hypoglycaemic[12] activities. In order to further optimize the clarification of its chemical compositions, especially diterpenes, and discover the bioactive components, from its extract we isolated two labdane diterpenes for the first time. Furthermore, we explored the anti-inflammatory effect of the two compounds. Herein, we described the isolation, structural elucidation as well as anti-inflammatory activity of the compounds.

MATERIALS AND METHODS

General

Optical rotations were measured on a Jasco P-1020 polarimeter at 25°C. UV spectra were performed on a Jasco V-550 UV/VIS spectrophotometer. IR spectra were established on a Jasco FI/IR-480 plus spectrometer. NMR spectra were performed on Bruker AV 500 equipment. Chemical shifts were revealed in ppm (δ) with tetramethylsilane (TMS) as an internal standard. HR-ESI-MS spectra were demonstrated on Agilent 6210 ESI/TOF mass spectrometer. HPLC was operated on an Agilent 1260 system including a G1329B pump and a G1315D UV detector, accompanied by a cosmosil C18 column (4.6 × 250 mm, 5 mm). As for Column chromatographies, silica gel (200–300 mesh, Qingdao Marine Chemical Factory), ODS (YMC, Tokyo) were employed. TLC analysis was implemented with precoated silica gel GF_{254} plates (Yantai Chemical Industry Research Institute). All of the solvents used in column chromatography and HPLC were of analytical grade (Tianjin Damao Chemical Plant) and chromatographic grade (Oceanpak, Sweden), respectively.

Plant material

The rhizomes of *Alpinia officinarum* Hance were collected from Guangdong Province, China, in January 2013 and identified by Prof. Guang-Xiong Zhou

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in Jinan University, Guangzhou, China. A voucher specimen (No. 20130101) was deposited at the Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou, China.

Isolation

Air-dried rhizomes (20 kg) of *Alpinia officinarum* Hance were first cut into pieces, and extracted with EtOH-H_2O (95%, v/v) for three times (3 × 75 L, 48 h for each) at room temperature. The solvent was removed by evaporating under reduced pressure to obtain a brown residue. The crude extract (3.7 kg) was then suspended in H_2O and fractioned with petroleum ether (3 × 3 L), EtOAc (3 × 3 L) and *n*-BuOH (3 × 3 L) successively. The petroleum ether soluble part was subjected to a silica gel column and eluted using a petroleum ether-EtOAc solvent system (100:0–0:100, v/v) to afford eighteen fractions (Fr.1-Fr.18).Fr.14 (27.0 g) were then separated by an ODS column to give 11 subfractions (Fr.14a-Fr. 14 k) using a MeOH-H_2O (10–95%, v/v) gradient as the mobile phase. From Fr. 14i evolved a mixed yellow solid mainly consist of compound **1** and compound **2** and the mixture was further purified by preparative HPLC, leading to the isolation of **1** (15.1 mg) and **2** (13.5 mg).

Cell culture

RAW 264.7 cells were grown with DMEM medium (GIBCO Inc, NY, USA), supplemented with 10% FBS, 2 mM L-glutamine, penicillin (100 U/mL), and streptomycin (100 μg/mL). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Cell viability assay

RAW 264.7 cells were seeded in 96-well plates with a density of 2 × 10^5/ml for 24 h, and treated with a series of concentrations of compound **1** and **2** for 2 h, followed by exposure to LPS of 1 μg/ml. After LPS treatment for another 24 h, MTT (St. Louis, MO, USA) was added to each well and further incubated for 4 h. The medium was then removed and 100 ml of dimethyl sulfoxide (DMSO) was added. The absorbance of each well was measured at 570 nm utilizing an automatic microplate reader (Amersham Pharmacia Biotech, NY, USA)[15–16]. Results were expressed as percentages of control group.

Determination of reactive oxide species generation

To measure intracellular hydrogen peroxide (H_2O_2) and superoxide anion (**(**$O2^{\bullet-}$) generation, RAW 264.7 cells were pretreated with the two compounds for 2 h and then were incubated with LPS (1 μg/ml) for 24 h[17]. After LPS induction, the cells were washed twice with HBSS (pH 7.4) and fluorescent probe (H_2DCF-DA or DHE) was added, respectively, to react with correspondent free radical (hydrogen peroxide, superoxide anion). The fluorescence intensity was tested immediately using an automatic microplate reader (BioTek Synergy Corporation, USA).

Statistical analysis

Data are expressed as the means ± SD from three independent experiments. Student's *t*-test was used to perform statistical analysis. Value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Compound **1** was obtained as white amorphous powder and its molecular formula was determined to be $C_{20}H_{30}O_2$ on the basis of $[M + H]^+$ signalat m/z 303.1704 (calcd for $C_{20}H_{31}O_2$, 303.1710) displayed in HR-ESI-MS spectrum. The UV spectrum of compound **1** showed absorption maxima at 206 nm. The IR absorption indicated the existence of hydroxyl (3447 cm^{-1}) and double bond (1647 cm^{-1}). In the ^1H and ^{13}C NMR spectraof **1**, a group of characteristic signals of labdane diterpenene could be observed, including signals for a furan ring [δ_H7.50 (^1H, t, J = 2.0 Hz, 4.41 (^1H, m), 6.47 (H, d, J = 1.5Hz) and δ_C 144.5, 140.9, 131.2, 110.1], for an exocyclic methylene [δ_H 4.88 (^1H, q, J = 1.5 Hz) and 4.76 (^1H, m); δ_C 107.5], for two methyl [δ_H0.85 (3H, s); δ_C34.4 and 0.81 (3H, s); δ_C22.6] and for an angular methyl [δ_H0.73 (3H, s); δ_C 15.6]. In addition, signals ascribable to an oxymethine [δ_H4.63 (^1H, dd, J = 9.6, 4.4 Hz); δ_C66.1] were also present. All the evidence above suggested a skeleton of labdane diterpene. Then by comparing the NMR values with those in literature, compound **1** was established as (12*S*)-15,16-epoxy-8(17),13(16),14-labdatrien-12-ol[13]. It was isolated and identified from *Alpinia officinarum* Hance for the first time.

Compound **2** was obtained as white amorphous powder. The HR-ESI-MS spectrum exhibited $[M + H]^+$ signal at m/z 303.2328 (calcd for $C_{20}H_{31}O_2$, 303.2324), suggesting a molecular formula of $C_{20}H_{30}O_2$. The UV spectrum of compound **2** also displayed absorption maxima at 206 nm. The IR absorption pointed the presence of hydroxyl (3447 cm^{-1}), carbonyl (1753 cm^{-1}) and double bond (1673 cm^{-1}). Similar to **1,** the ^1H and ^{13}C NMR spectra of **2** implied a labdane diterpene structure including signals of an α,β-unsaturated δ-lactone moiety [δ_H6.55 (^1H, m); δ_C171.4, 141.2, 126.6], an exocyclic methylene [δ_H 4.84 (^1H, q, J = 1.0 Hz), 4.48 (^1H, q, J = 1.0 Hz); δ_C149.3, 108.0], two methyl [δ_H0.90 (3H, s); δ_C34.0; 0.84 (3H, s); δ_C22.2] and an angular methyl [δ_H0.76 (3H, s); δ_C14.8]. In the HMBC spectrum, correlations between H-12 (δ_H6.55) and C-14 (δ_C25.8)/C-16 (δ_C171.3), H-11 (δ_H2.44, 2.28) and C-13 (δ_C126.6) were observed, suggesting the lactone moiety was linked

Table 1 ^{1}H and ^{13}C NMR spectral data of 1 and 2 (δ in ppm, J in Hz, C_2D_3N)[a].

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
1	1.75 (m) 0.86 (m)	40.1	1.77 (m) 1.15 (td, 13.0, 3.5)	39.8
2	1.57 (tt, 14.0, 3.2) 1.47 (m)	20.5	1.62 (m) 1.51 (m)	20.1
3	1.38 (m) 1.13 (td, 13.2, 4.0)	43.3	1.43 (m) 1.24 (d, 2.5)	42.8
4		34.6		34.2
5	1.03 (td, 12.4, 2.8)	56.7	1.20 (dd, 12.5, 2.5)	56.0
6	1.74 (m) 1.37 (m)	25.6	1.75 (m) 1.35 (dd, 13.0, 4.5)	25.0
7	2.40 (dq, 12.4, 2.4) 1.93 (m)	39.4	2.40 (m) 2.06 (m)	38.6
8		150.2		149.3
9	1.46 (m)	54.0	1.96 (d, 11.0)	57.1
10		40.6		40.0
11	1.92 (m)	34.0	2.44 (m) 2.28 (m)	25.9
12	4.63 (dd, 9.6, 4.4)	66.1	6.55 (m)	141.2
13		131.2		126.6
14	6.47 (d, 1.5)	110.1	2.95 (m)	25.8
15	7.50 (t, 2.0)	144.5	4.35 (td, 7.5, 0.5)	66.0
16	7.41 (m)	140.9		171.3
17	4.88 (q, 1.5) 4.76 (m)	107.5	4.84 (q, 1.0) 4.48 (q, 1.0)	108.0
18	0.85 (s)	34.4	0.90 (s)	34.0
19	0.81 (s)	22.6	0.84 (s)	22.2
20	0.73 (s)	15.6	0.76 (s)	14.8

[a]Overlapped signals are reported without designating multiplicity.

to the labdane skeleton via a C=C bond at C-11 and C-12. By comparing the NMR data with the values in the literature, compound **2** was determined to be (12*E*)-labda-8(17),12(13)-dien-15,16-olide[14]. It was also obtained from this plant for the first time.

The protective effects for RAW 264.7 cells from LPS damage of the two compounds were investigated. As

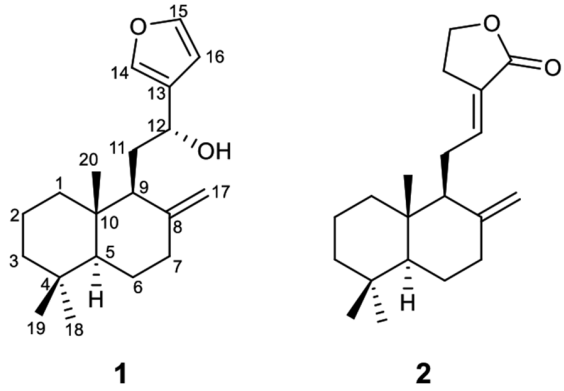

Fig.1 Chemical structures of 1 and 2.

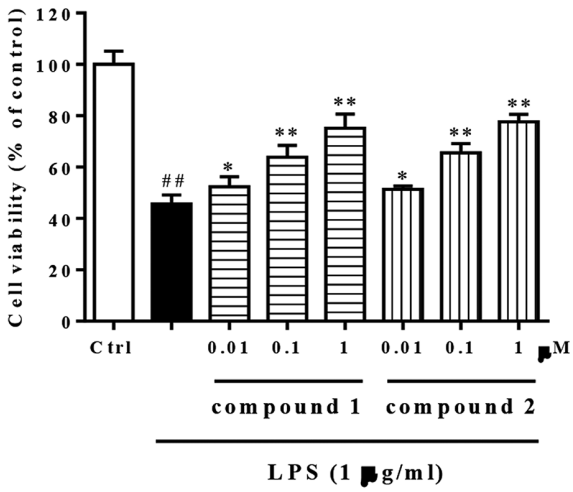

Fig. 2 Effect of compound 1 and 2 against LPS damage. Cell viability was tested by the MTT assays. $^{\#\#}P < 0.001$ vs control group; $^{*}P < 0.05$ and $^{**}P < 0.01$ vs LPS treated group.

shown in Fig. 2, LPS treatment caused about 55% cell damage compared to the untreated cells. However, both compounds **1** and **2** at different concentration can significantly prevent RAW 264.7 cells from LPS damage in a dose-dependent manners.

To evaluate the antioxidant activity of the **1** and **2**, intracellular ROS was measured. When treated with LPS, both H_2O_2 and $O2^{\bullet-}$ levels were greatly increased compared to the control group. However, in the cells treated with **1** or **2**, the H_2O_2 and $O2^{\bullet-}$ levels were decreased significantly.

CONCLUSION

Two labdane diterpenes, (12*S*)-15,16-epoxy-8(17), 13(16), 14-labdatrien-12-ol (**1**) and (12*E*)-labda-8(17),12(13)-dien-15,16-olide (**2**), which were rarely natural originated, were isolated and identified from the rhizomes of *Alpinia officinarum*. Not only did they enriched the chemical constituents of this plant, but also laid a solid foundation for its pharmacological research. In our research both of them exhibit strong anti-inflammatory effect and antioxidant activity in vitro, which may be related to the inhibition of ROS.

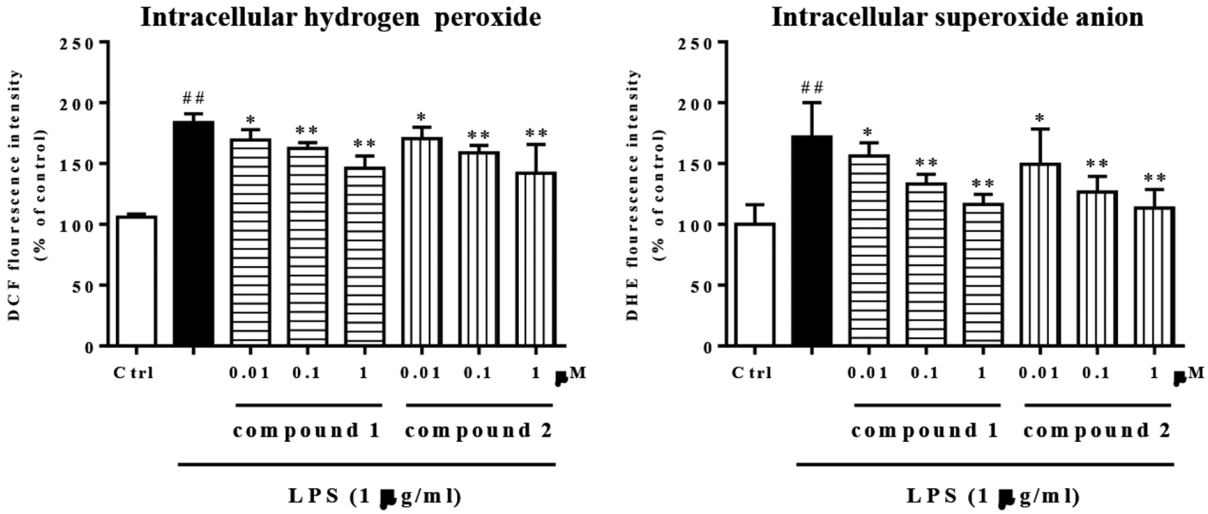

Fig. 3 Compound 1 and 2 attenuates the reactive oxygen species induced by LPS. (A) Intracellular hydrogen peroxide was measured by DCF-DA. (B) Intracellular superoxide anion was measured by DHE. Date were presented as the increased ratio of relative fluorescence intensity compared to control. The results were the mean ± SD of three independent experiments. $^{\#\#}P < 0.001$ vs control group; $^{*}P < 0.05$ and $^{**}P < 0.01$ vs LPS treated group.

REFERENCES

1. The State Pharmacopoeia Commission of the People's Republic of China. Pharmacopoeia of the People's Republic of China, Chemical Industry Press, Beijing, China, 2005; p. 202.
2. Tao L, Wang ZT, Zhu EY, Lu YH, Wei DZ. HPLC analysis of bioactive flavonoids from the rhizome of *Alpiniaofficinarum*. S Afr J Bot. 2006;72(1):163–166.
3. Itokawa H, Morita M, Mihashi S. Two new diarylheptanoids from *Alpinia officinarum* Hance. Chem Pharm Bull. 1981;29(8): 2383–2385.
4. Uehara SI, Yasuda I, Akiyama K, Morita H, Takeya K, Itokawa H. Diarylheptanoids from the rhizomes of *Curcuma xanthorrhiza* and *Alpinia officinarum*. Chem Pharm Bulletin. 1987;35(8): 3298–3304.
5. Nan P, Hu Y, Zhao J, Feng Y, Zhong Y. Chemical composition of the essential oils of two Alpinia species from Hainan Island, China. Z Naturforsch C. 2004;59(3–4):157–160.
6. Tram NL, Ryo Y, Koji K. Volatile components of the essential oils in Galanga (*Alpinia officinarum* Hance) from Vietnam. Food Sci Technol Res. 2001;7(4):303–306.
7. Yasukawa K, Sun Y, Kitanaka S, Tomizawa N, Miura M, Motohashi S. Inhibitory effect of the rhizomes of *Alpinia officinarum* on TPA-induced inflammation and tumor promotion in two-stage carcinogenesis in mouse skin. J Nat Med. 2008;62(3):374–378.
8. Shen J, Zhang H, Xu B, Pan JX. The antioxidative constituents of rhizomes of *Alpinia officinarum*. Nat Prod Res Dev. 1997;10(2): 33–36.
9. Konno K, Sawamura R, Sun Y, Yasukawa K, Shimizu T, Watanabe W, et al. Antiviral activities of diarylheptanoids isolated from *Alpinia officinarum* against respiratory syncytial virus, poliovirus, measles virus, and herpes simplex virus type 1 *in vitro*. Nat Prod Commun. 2011;6(12):1881–1884.
10. Shin D, Kinoshita K, Koyama K, Kunio T. Antiemetic principles of *Alpinia officinarum*. J Nat Prod. 2002;65(9):1315–1318.
11. An N, Zou ZM, Tian Z, Luo XZ, Yang SL, Xu LZ. Diarylheptanoids from the rhizomes of *Alpinia officinarum* and their anticancer activity. Fitoterapia. 2008;79(1):27–31.
12. Akhtar MS, Khan MA, Malik MT. Hypoglycaemic activity of *Alpiniagalanga* rhizome and its extracts in rabbits. Fitoterapia. 2002;73(7):623–628.
13. Giang PM, Otsuka H, Son PT. A furnano labdane diterpene alcohol from *Alpinia tonkinensis* Gagnep. J Chem. 2005;43(3): 375–378.
14. Chimnoi N, Pisutjaroenpong S, Ngiwsara L, Dechtrirut D, Chokchaichamnankit D, Khunnawutmanotham N, et al. Labdane diterpenes from the rhizomes of *Hedychium coronarium*. Nat Prod Res. 2008;22(14):1249–1256.
15. Shih MF, Cheng YD, Shen CR, Cherng JY. A molecular pharmacology study into the anti-inflammatory actions of Euphorbia hirta L. on the LPS-induced RAW 264.7 cells through selective iNOS protein inhibition. J Nat Med. 2010;64(3):330–335.
16. Reddy DB, Reddanna P. Chebulagic acid (CA) attenuates LPS-induced inflammation by suppressing NF-κB and MAPK activation in RAW 264.7 macrophages. Biochem Biophys Res Comm. 2009;381(1):112–117.
17. Lin HC, Su SL, Lu CY, Lin AH, Lin WC, Liu CS, et al. Andrographolide inhibits hypoxia-induced HIF-1α-driven endothelin 1 secretion by activating Nrf2/HO-1 and promoting the expression of prolyl hydroxylases 2/3 in human endothelial cells. Environ Toxicol. 2016; doi: 10.1002/tox.22293