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Natural nitric oxide (NO) inhibitors from *Chloranthus japonicus*

Yan-Qiong Guo †, Jing-Jun Zhao †, Zhen-Zhen Li, Gui-Hua Tang, Zhi-Min Zhao, Sheng Yin *

School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, Guangdong 510006, PR China

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ABSTRACT

Eight new lindenane sesquiterpenoid dimers, chlojapolides A–H (**1**–**8**), along with 11 known analogues were isolated from the whole plant of *Chloranthus japonicus*. Their structures including absolute configurations were elucidated by spectral and chemical methods. All the compounds were examined for their inhibitory effects on the nitric oxide (NO) production induced by lipopolysaccharide (LPS) in RAW 264.7 macrophages, and compounds **1**, **11**, **13**, and **17** exhibited pronounced inhibition with IC_{50} values in the range of 6.91–15.75 μM, being more active than the positive control, quercetin (IC_{50} = 15.90 μM).

Inflammation is generally considered as an essentially protective response to tissue injury caused by noxious physical, chemical or microbiological stimulus.[1] Nitric oxide (NO), a highly reactive radical, is a key mediator or regulator in inflammatory responses.[2] Activation of macrophage cells and consequent over expression of NO could result in numerous oxidations and cause the dysfunctional cellular processes, cell signaling pathway disruption, and even the tissue damage.[3,4] There are numerous evidences suggesting that suppression of NO production in macrophages was a key anti-inflammatory approach and may provide a strategy for drug development.

Chloranthus japonicus Sieb. (Chloranthaceae), a perennial herbaceous plant growing in southern China, has been applied in the Traditional Chinese Medicine (TCM) for the treatment of inflammation-related diseases such as rheumatic arthralgia, bone fracture, and pneumonia.[5] Previous investigations on this plant have proved that it was a rich source of structurally diverse lindenane dimers,[6,7] some of which exhibited inhibition of the expression of cell adhesion molecules[8] and AMPK-dependent lipid content,[9] immunotoxicity,[10] and anti-HIV-1 activities.[11] In our efforts toward novel nitric oxide (NO) inhibitors from medicinal plants,[12,13] a novel 1,3-dioxolane linked-lindenane dimer, chlojapolactone A, was isolated from a low polar fraction of the ethanolic extract of *C. japonicus*.[14] Continuing excavation of high polar fractions of the extract led to the isolation of eight new lindenane sesquiterpenoid dimers and 11 known analogues. Bioassay verified that compounds **1**, **11**, **13**, and **17** were potent NO inhibitors with IC_{50} values ranging from 6.91 to 15.75 μM. Herein, details of the isolation, structural elucidation, and NO inhibitory activities of these compounds are described.

The air-dried powder of the whole plant of *C. japonicus* (1.0 kg) was extracted with 95% EtOH at room temperature to give a crude extract, which was suspended in H_2O and successively partitioned with petroleum ether, EtOAc, and *n*-BuOH. Various column chromatographic separations of the EtOAc extract afforded compounds **1**–**19** (Fig. 1).

Compound **1**, a white powder, had the molecular formula $C_{40}H_{46}O_{13}$, as determined by HRESIMS ion at *m*/*z* 757.2817 $[M+Na]^+$ (calcd 757.2831). Its ^1H NMR spectrum showed an oxymethine [δ_H 3.92 (1H, s)], two tertiary methyls [δ_H 1.00 and 0.85 (each 3H, s)], a olefinic methyl [δ_H 1.87 (3H, s)], a methoxyl [δ_H 3.68 (3H, s)], a tiglate moiety [δ_H 6.88 (1H, q, J = 6.6 Hz, H-3‴), 1.82 (3H, d, J = 6.6 Hz, H-4‴), and 1.83 (3H, s, H-5‴)], and two highly upfield-shifted protons [δ_H 0.70 and 0.27 (each 1H, m)] characterized for two cyclopropane rings of lindenane sesquiterpenoids.[11,15–17] The ^{13}C NMR spectrum, in combination with DEPT experiments, resolved 40 carbon resonances attributable to six carbonyls, eight olefinic carbons (one trisubstituted and three tetrasubstituted double bonds), six methyls (one methoxyl), eight sp^3 methylenes (two oxygenated), eight sp^3 methines (one bearing heteroatom), and four sp^3 quaternary carbons (two oxygenated). The above mentioned information showed high similarity to those of *co*-isolated known sesquiterpenoid dimer shizukaol C (**16**),[18] except for the presence of an additional succinyl group. The key HMBC correlation from H_2-13′ to the carbonyl (δ_C 172.0, C-1″) of the succinyl group revealed that **1** was a 13′-*O*-succinyl derivative of **16**. Detailed 2D NMR analyses (HSQC, ^1H–^1H

* Corresponding author.
† These authors contribute equally.

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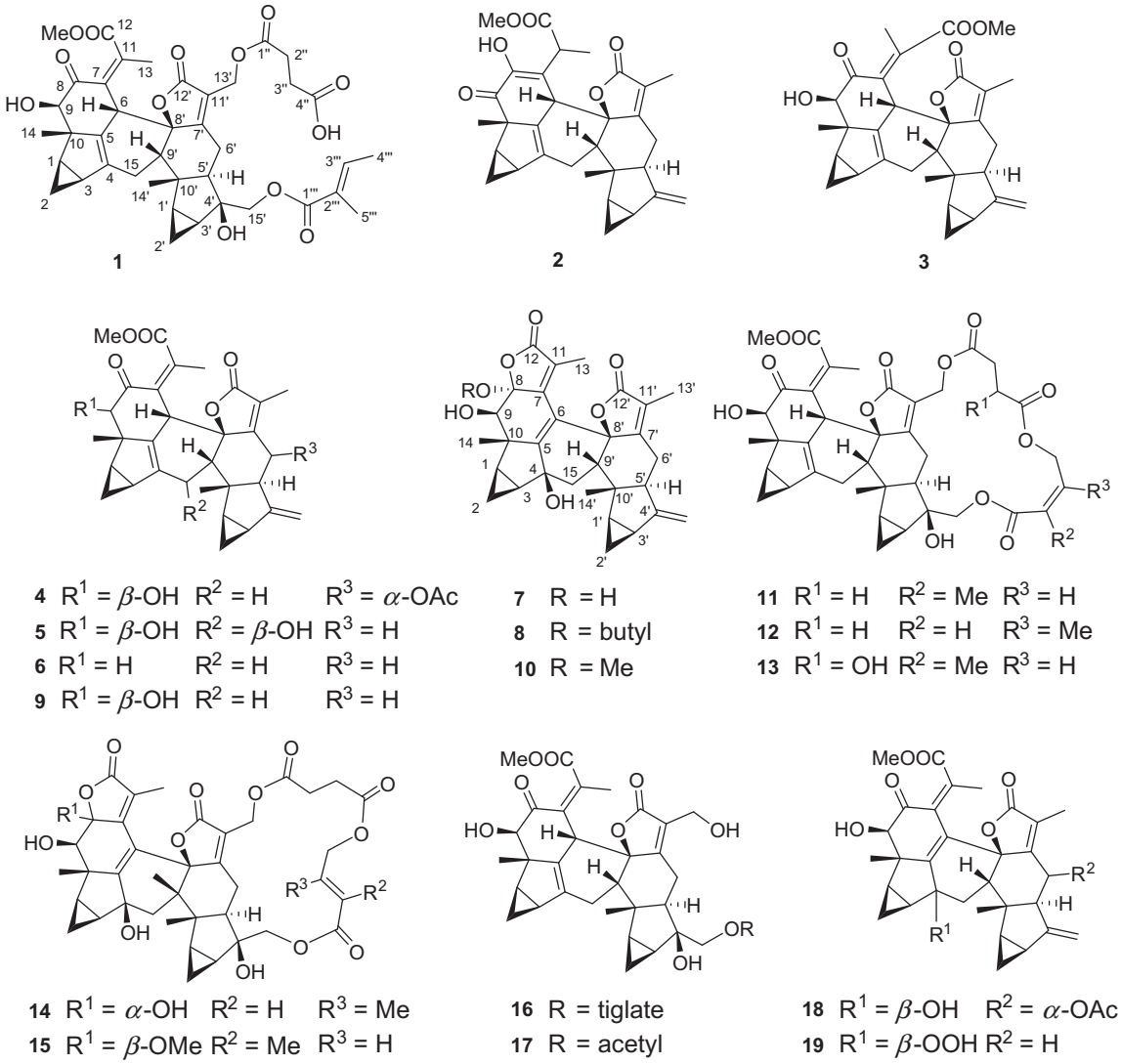

Figure 1. Structures of compounds **1**–**19**.

COSY, and HMBC) supported the planar structure of **1** as depicted (Fig. 2). The relative configuration of **1** was assigned to be the same as that of **16** by NOESY experiment and comparison of their 1D NMR data. Compound **1** was given a trivial name chlojapolide A.

Compound **2**, a yellow powder, had the molecular formula $C_{31}H_{34}O_6$, as determined by HRESIMS ion at *m*/*z* 525.2261 [M +Na]$^+$ (calcd 525.2248). Its ^{1}H and ^{13}C NMR data were quite similar to those of the *co*-isolated known sesquiterpenoid dimer shizukaol A (**9**).[19] The main differences were due to the replacement of an oxygenated methine and a olefinic methyl in **9** by a upfield methine and a doublet methyl in **2**, indicating the migration of α, β-unsaturated ketone from C_7=C_{11}–C_8=O in **9** to C_7–C_8=C_9=O in **2**. This was further confirmed by the ^{1}H–^{1}H COSY correlation of H_3-13/H-11 and the HMBC correlations of H-11/C-7 and C-8, H-6/C-7 and C-8, and H_3-14/C-9 (Supplementary data, S89). Thus, the gross structure of **2** was determined as depicted. The relative configuration of **2** was assigned to be the same as that of **9** by the NOESY experiment and comparison of their 1D NMR data. The configuration of C-11 was left unassigned due to the rotatable C-7–C-11 bond. Compound **2** was named as chlojapolide B.

Compound **3** showed the molecular formula of $C_{31}H_{34}O_6$ as deduced by HRESIMS. The ^{1}H and ^{13}C NMR data of **3** showed high similarity to those of **9**[19] with the notable differences arisen from the $\Delta^{7(11)}$ region. In comparison with those of **9**, the ^{13}C NMR

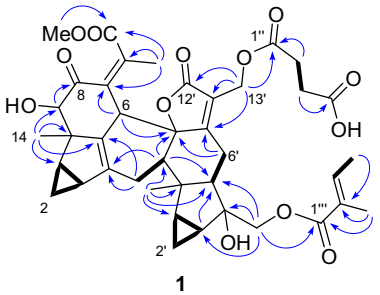

Figure 2. ^{1}H–^{1}H COSY (▬) and key HMBC (⌒) correlations of compound **1**.

chemical shifts of C-7, C-12 and C-13 in **3** were downfield shifted (3.4, 1.5, and 1.0 ppm, respectively), while those for C-6, C-8 and C-11 were upfield shifted (1.0, 4.1, and 3.8 ppm, respectively), revealing that the geometry for $\Delta^{7(11)}$ was *E* in **3**.[20] The key NOESY correlations of MeO-12/H-6 and H-13′ further confirmed the *E*-$\Delta^{7(11)}$. Detailed 2D NMR analyses (HSQC, ^{1}H–^{1}H COSY, and HMBC) supported the structure of **3** as depicted. The remaining relative configuration of **3** was determined to be the same as **9** by NOESY experiment and comparison of the 1D NMR data. Compound **3** was given the name chlojapolide C.