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Daphnane diterpenoids with nitric oxide inhibitory activities and interactions with iNOS from the leaves of *Trigonostemon thyrsoideus*

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ABSTRACT

A phytochemical investigation to search for new nitric oxide (NO) inhibitors resulted in the isolation of seven previously undescribed daphnane diterpenoids, thyrsoidpenes A–G, from the leaves of *Trigonostemon thyrsoideus*. Their structures including absolute configurations were elucidated on the basis of extensive NMR spectroscopic data analysis and the time-dependent density functional theory (TDDFT) electronic circular dichroism (ECD) calculations. Thyrsoidpenes B–G feature rare polycyclic caged structures of daphnane diterpenoid orthoester. The NO inhibitory effects were examined and all of the compounds showed inhibitory activities toward LPS-induced NO production in murine microglial BV-2 cells. The possible mechanism of NO inhibition of some bioactive compounds was also investigated using molecular docking, which revealed the interactions of bioactive compounds with the iNOS protein.

1. Introduction

The genus *Trigonostemon*, a member of the Euphorbiaceae plant family, contains about 50 species distributed mainly in the tropical and subtropical regions of Asia (Editorial Committee of the Flora of China, 1996). There are about 10 species growing in South China. Some *Trigonostemon* species, such as *T. thyrsoideus*, *T. howii*, *T. lutescens*, and *T. chinensis,* have been used historically as folk medicines for the treatment of asthma, diarrhea, and skin diseases in China and Thailand (Xu and Yue, 2014). Diterpenoids, triterpenoids, alkaloids, phenolics, and steroids have been reported to be the main components of this genus, which exhibited various bioactivities, such as cytotoxic, anti-leukemic, insecticidal, and antimicrobial effects (Chen et al., 2011; Ma et al., 2013; Xu and Yue, 2014; Li et al., 2014; Tan et al., 2015; Yang et al., 2015, 2016; Liu et al., 2016; Bourjot et al., 2014; Xu et al., 2016b; Kaemchantuek et al., 2017; Wang et al., 2017b). *Trigonostemon thyrsoideus* Stapf is a shrub or small tree growing mainly in Yunnan, Guangxi, and Guizhou provinces of China (Editorial Committee of the Flora of China, 1996). In our ongoing search for new nitric oxide (NO) inhibitors as anti-inflammatory agents (Xu et al., 2016c; Wang et al., 2017a), the chemical constituents of the leaves of *T. thyrsoideus* were investigated. Seven previously undescribed diterpenoids (Fig. 1), designated as thyrsoidpenes A–G (**1**–**7**), were isolated from the methanol extract of the leaves of *T. thyrsoideus*. The structures including absolute configurations were established on the basis of extensive NMR spectroscopic data analysis and comparison of experimental and calculated electronic circular dichroism (ECD) spectra. Compounds **2**–**7** possess rare polycyclic caged structures of daphnane diterpenoid orthoester. In this paper, we describe the structural determination and NO inhibitory effects of these isolated daphnane diterpenoids as well as their interactions with the iNOS protein.

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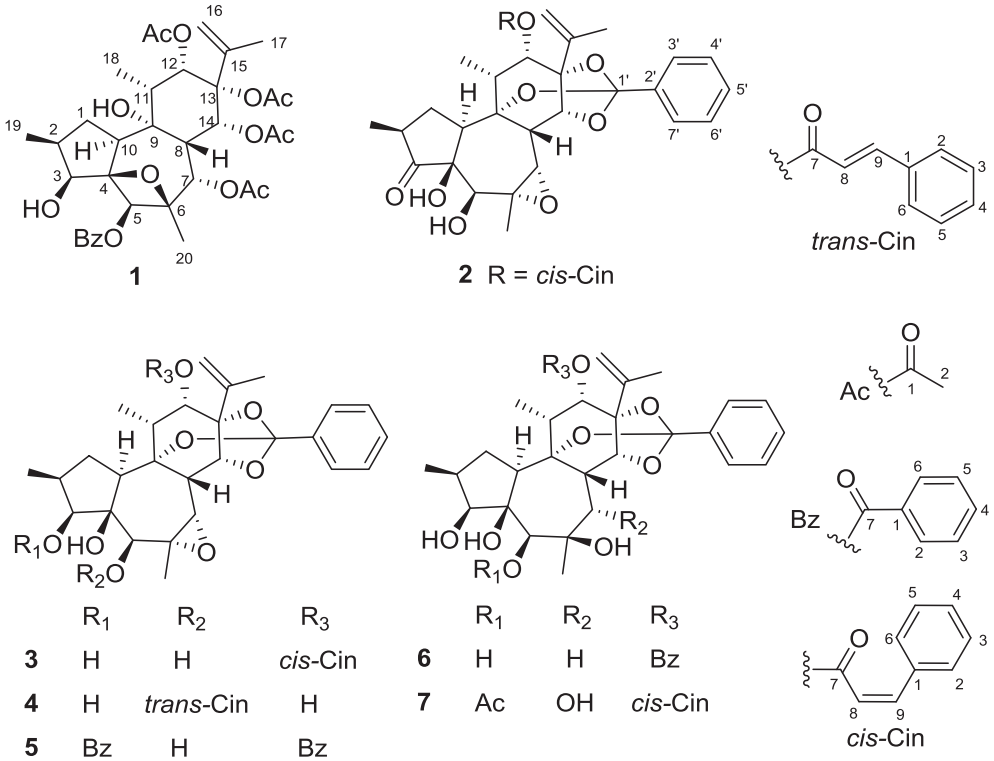

Fig. 1. Structures of compounds **1**–**7**.

2. Results and discussion

2.1. Structural elucidation

Compound **1** was obtained as an amorphous powder. Its molecular formula was determined to be $C_{35}H_{44}O_{13}$ through the presence of a HRESIMS ion at *m*/*z* 695.2678 $[M + Na]^+$ (calcd for $C_{35}H_{44}NaO_{13}$, 695.2680) and the ^{13}C NMR data (Tables 1 and 2). From the ^1H and ^{13}C NMR spectra, the methyl singlets (δ_H 2.19, 2.14, 2.13, and 1.89) and the corresponding carbon signals at δ_C 169.3, 20.9, 167.9, 21.3, 170.1, 21.3, 168.4, and 21.4, revealed the presence of four acetyloxy groups; and the carbon resonances (δ_C 165.9, 129.5, 129.9 × 2, 128.6 × 2, and 133.4) and the corresponding aromatic proton signals [δ_H 8.12 (2H, t, J = 7.6 Hz), 7.46 (2H, d, J = 7.6 Hz), and 7.58 (1H, t, J = 7.6 Hz)] (Table 2) were indicative of one benzoyloxy group. Excluding the signals for the above acyloxy groups, the ^{13}C NMR spectrum showed additional 20 carbon resonances including two olefinic and nine oxygenated carbons (Table 1). The remaining nine aliphatic carbons were assigned as four methyls [δ_C 20.1 (C-17), 11.6 (C-18), 15.5 (C-19), and 19.7 (C-20)], one methylene [δ_C 34.9 (C-1)], and four methines [δ_C 32.7 (C-2), 39.2 (C-8), 49.5 (C-10), and 39.9 (C-11)] with the aid of DEPT and HMQC spectra (Table 1). These spectroscopic features suggested compound **1** is a daphnane-type diterpenoid derivative (Zhang et al., 2010a; Chen et al., 2010a; Cheng et al., 2013; Li et al., 2013; Liu et al., 2015), which was supported by the following HMBC and ^1H-^1H COSY experiments (Fig. 2). Upon comparison of its NMR data with those of diterpenoids reported from the genus *Trigonostemon*, a highly oxygenated daphnane-type diterpenoid skeleton for **1** was obvious. The subsequent interpretation of HMBC and ^1H-^1H COSY spectra verified this skeletal type of daphnane-type diterpenoid, and the oxygenated and olefinic skeletal carbons at δ_C 72.1 (C-3), 92.7 (C-4), 73.6 (C-5), 84.8 (C-6), 78.9 (C-7), 76.6 (C-9), 72.7 (C-12), 80.9 (C-13), 75.2 (C-14), 139.6 (C-15) and 119.4 (C-16) were assigned. Further analysis of 1D and 2D NMR spectra allowed all of the skeletal protons and carbons to be assigned (Tables 1 and 2). The positions of the acyloxy groups were determined from the HMBC spectrum. The benzoyloxy and three acetyloxy groups were deduced to be located at C-5, C-7, C-12, and C-14, respectively, by the corresponding HMBC correlations of skeletal oxymethine protons to the corresponding carbonyl carbons (Fig. 2). The residual one acetyloxy group was inferred to be located at C-13, which was supported by the downfield shift of C-13 (Li et al., 2013; Cheng et al., 2013). Hitherto, the planar structure seemed to be established. However, the molecular formula of this planar structure differed from that suggested by the HRESIMS data, indicating the presence of one more ring according to the index of hydrogen deficiency. The chemical shifts of C-4 (δ_C 92.7) and C-6 (δ_C 84.8) and the HRESIMS data of compound **1** strongly pointed toward a C-4–*O*–C-6 bridge linkage (Zhang et al., 2010b; Chen et al., 2010b). Thus, the planar structure of compound **1** was determined.

The configuration of compound **1** was elucidated by the NOESY spectrum and Chem3D modeling (Fig. 3). The NOESY correlations of H-8/H-1β, H_3-19/H-1β, H-8/H-7, H-8/H-14, H-8/H-11, H-8/H_2-16, H-14/H_2-16, H_3-17/H-12, OH-9/H-10, H-10/H-3, H-5/OH-9, and H-2/H-10, together with Chem3D modeling, suggested a molecular conformation as depicted in Fig. 3, in which H-2, H-3, H-5, H-7, H-8, OH-9, H-11, H-12, H-14, and the C-13 acetyloxy group was assigned as α-, α-, α-, β-, β-, α-, β-, β-, β-, and α-oriented and the C-4–*O*–C-6 bridge was on the β-face. The absolute configuration of **1** was established by the time-dependent density functional theory (TDDFT) ECD calculations (Li et al., 2010; Xu et al., 2016a). Starting from the conformation of **1** deduced from the NOESY correlations and Chem3D modeling, conformational searches with the MMFF94S force field by MOE software and geometry optimizations by the Gaussian 09 package were performed (Frisch et al., 2010). Then, the ECD spectra were calculated at the CAM-B3LYP/SVP level with the CPCM model in acetonitrile. The obtained ECD spectrum of