Phytochemistry 155 (2018) 107–113

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Dimeric- and trimeric sesquiterpenes from the flower of *Inula japonica*

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ARTICLE INFO

Keywords:
Inula japonica
Asteraceae
Sesquiterpene trimer
Sesquiterpenes dimer
Cytotoxic activity
Nitric oxide

ABSTRACT

An undescribed unusual sesquiterpene trimer and three sesquiterpene dimers were isolated from the flowers of *Inula japonica*. Their structures were elucidated by extensive analysis of 1D and 2D NMR spectroscopic data as well as HRESIMS data. Inulajaponicolide A has an undescribed carbon skeleton comprising of one xanthanolide and two guaianolide units with the linkage mode of C-11/C-3′ and C-11′/C-1″ via a Diels-Alder cycloaddition reaction. Inulajaponicolides C and D exhibited moderate cytotoxic activity against A 549 and NCI-H460 human cancer cell lines with IC_{50} values ranging from 8.5 to 17.8 μM. Inulajaponicolides A-D and linearifolianoid A possessed significant inhibitory potency against nitric oxide production in LPS-induced RAW264.7 cells with IC_{50} values ranging from 1.0 to 4.1 μM.

1. Introduction

Inula japonica Thunb. (Asteraceae) is mainly distributed in Korea, mainland China, and Japan. The flower of this plant has been used to treat tracheitis, bronchitis, digestive disorders, and inflammation (Tang and Eisenbrand, 2011). Previous phytochemical studies of *I. japonica* have reported the isolation of sesquiterpenes, especially sesquiterpene lactone monomers and dimers, which possessed anti-inflammatory, anti-tumor, anti-diabetic, and anti-bacterial effects (Jin et al., 2016; Qin et al., 2009, 2010; 2011; Seca et al., 2015; Wang et al., 2014; Wu et al., 2016). As a part of a program to search for the plant-derived anti-cancer and anti-inflammatory compounds, four undescribed sesquiterpenes: one trimeric sesquiterpene (**1**), three dimeric sesquiterpenes (**2–4**), and one known compound, linearifolianoid A (**5**), were isolated from the flowers of *I. japonica* (Fig. 1). The structures of the compounds were elucidated by 1D and 2D NMR techniques including HSQC, HMBC, ^{1}H-^{1}H COSY and ROESY data. In this paper, we describe the isolation and structure elucidation of the undescribed compounds, cytotoxic activity as well as the inhibitory effects on LPS-induced nitric oxide (NO) production in RAW264.7 macrophages.

2. Results and discussion

Compound **1** was obtained as white amorphous powder. Its molecular formula, $C_{47}H_{58}O_9$, was deduced from the HRESIMS $[M + Na]^+$ ion at *m/z* 789.3966 (calcd 789.3973) and ^{13}C NMR data (Table 1), which indicated 19 indices of hydrogen deficiency. The ^{1}H NMR data of 1 (Table 1) showed the presence of four singlet methyl signals at δ_H 1.62 (H_3-15′), 1.73 (H_3-15″), 2.03 (OAc), and 2.15 (H_3-15), three doublet methyl signals at δ_H 1.02 (d, J = 7.5 Hz, H_3-14′), 1.03 (d, J = 7.5 Hz, H_3-14″), and 1.16 (d, J = 7.0 Hz, H_3-14), four oxygenated methine protons at δ_H 4.07 (tdd, J = 11.0, 9.5, 3.0 Hz, H-8′), 4.24 (m, H-8″), 4.48 (br s, H-2′), and 4.76 (tdd, J = 11.0, 5.5, 5.5 Hz, H-8), an olefinic proton at δ_H 5.53 (br d, J = 9.0 Hz, H-5), and an exomethylene at δ_H 5.48 (br d, J = 3.0 Hz, H-13″a) and 6.18 (br d, J = 3.0 Hz, H-13″b). The ^{13}C NMR data, associated with HSQC experiments, exhibited 47 carbon signals comprising fifteen quaternary carbons, thirteen methines, twelve methylenes, and seven methyl groups. The presence of an acetoxy group was identified by the corresponding ^{13}C NMR signals at δ_C 170.1 and 21.1. The HMBC (Fig. 2) cross-peaks between H-2′/OAc indicated that acetoxy group was attached at C-2′. A further analysis of 1D and 2D NMR data of the remaining 45 carbon signals indicated that they were assigned to the three different sesquiterpene moieties designated as units A, B, and C. With the characteristic signals of three set

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https://doi.org/10.1016/j.phytochem.2018.07.008
Received 13 March 2018; Received in revised form 26 June 2018; Accepted 18 July 2018

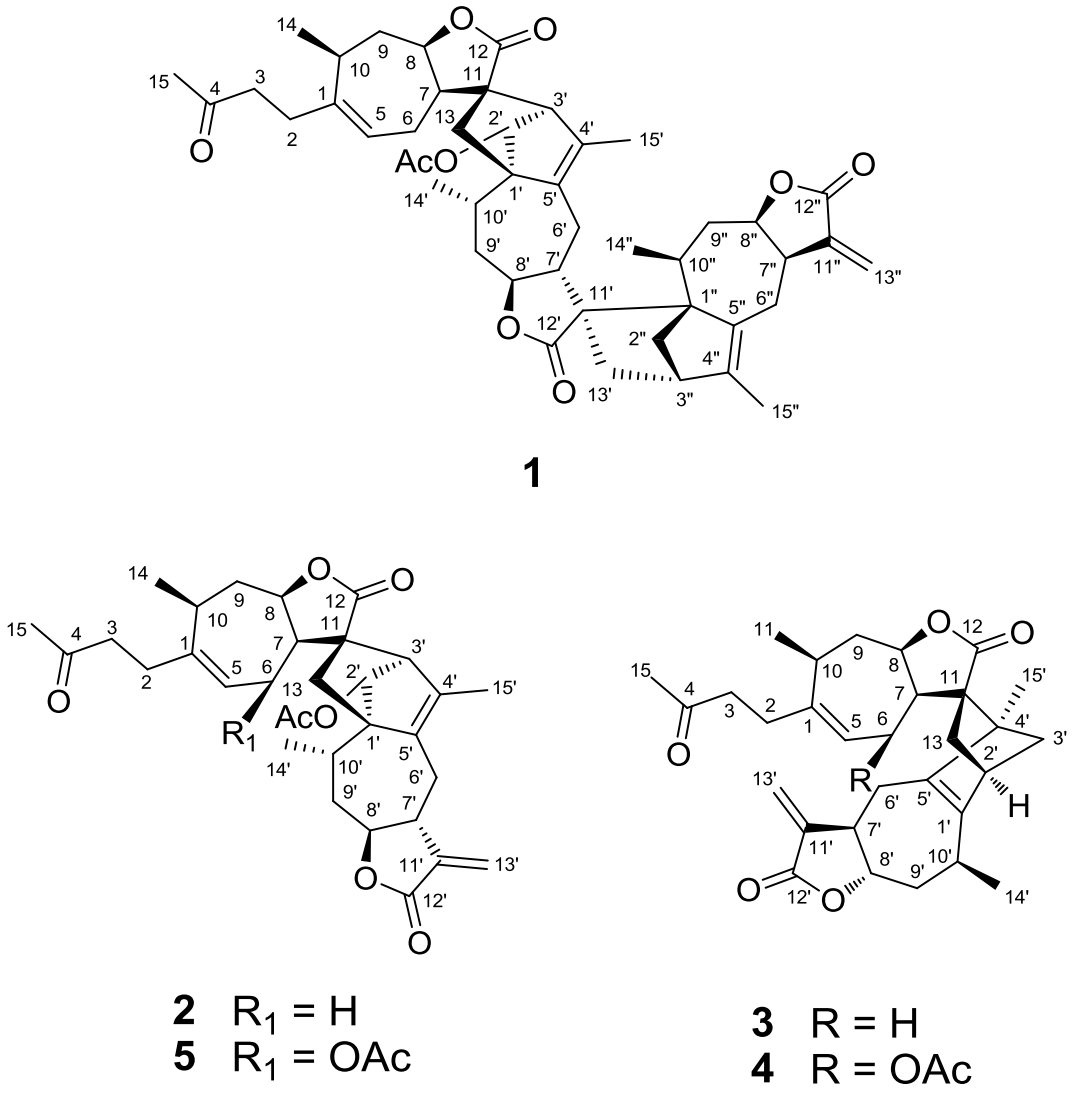

Fig. 1. Structures of compounds **1–5**.

of lactone groups at δ_C 178.8 (C-12), 182.5 (C-12′), and 170.4 (C-12″), it implied that compound **1** might be a trimeric sesquiterpene lactone. Compared with the spectroscopic data of known sesquiterpene dimers from the genus *Inula* (Chen et al., 2016; Jin et al., 2006; Qin et al., 2009, 2010, 2011, 2012), 1D and 2D NMR data suggested the presence of a xanthanolide in unit A and two guaianolides in units B and C. The HMBC correlations between H-8 to C-6, C-7, C-10, C-11, C-12; H-5 to C-2, C-7,C-10; H-14 to C-1, C-9, C-10; H_3-15 to C-3, C-4; H-7 to C-11, C-12, C-13, together with two proton-bearing chains of H_2-2/H_2-3 and H-5/H_2-6/H-7/H-8/H_2-9/H-10/H_3-14 revealed the existence of the xanthanolide unit A. Similarly, two units B and C were established as guaianolide moieties according to the ^{1}H-^{1}H correlations system H_2-6′/H-7′/H-8′/H_2-9′/H-10′/H_3-14′ and H-2′/H-3′; H_2-6″/H-7″/H-8″/H_2-9″/H-10″/H_3-14″ and H-2″/H-3″/H-13′, combined with the key HMBC correlations of H-8′/C-6′, C-7′, C-10′, C-11′, C-12′ and H_3-15′/C-3′, C-4′, C-5′; H-8″/C-6″, C-7″, C-10″, C-11″, C-12″ and H_3-15″/C-3″, C-4″, C-5″. The key ^{1}H-^{1}H COSY correlations of H-2′/H-3′ as well as the HMBC correlations from H_2-13 to C-11, C-12, C-2′, C-3′, and C-5′, H-2′ to C-11 and C-13, and H-3′ to C-7, C-13, C-2′, C-4′, and C-5′ clearly indicated that xanthanolide unit A and guaianolide unit B were connected via two C-C single bonds between C-11 and C-3′ and between C-13 and C-1′ (Fig. 2). The linkage of two guaianolide units B and C via two C-C single bonds between C-11′/C-1″ and C-13′/C-3″ was established by the ^{1}H-^{1}H COSY correlations of H_2-13′/H-3″/H_2-2″ and the HMBC correlations from H_2-13′ to C-11′, C-12′, C-7′, and C-4″, H-3″ to C-11′ and C-1″, and H_2-2″ to C-11′, C-1″, C-4″, and C-5″ (Fig. 2).

The relative configuration of **1** was further confirmed by the analysis of ROESY data, in which the key correlations of H-7/H-8/H-10/H-3′ indicated that H-7, H-8, H-10, and H-3′ were in the same face, and assigned as α-orientation. The absence of the correlations of H_3-14 with H-7 and H-8 in the ROESY spectrum of **1** further confirmed the above proposition. The ROESY correlations of H-2′/H-3′/H_3-14′/H-8′ suggested that H-2′, H-3′, H_3-14′, and H-8′ were assigned as α-orientation, while H-7′ was assigned as β-orientation. In addition, the correlations of H-8′/H-13′/H-3″/Ha-2″/H_3-14″ suggested that H-8′, H-13′, H-3″, and H-10″ were assigned as α-orientation, while H-2″ and H_3-14″ were assigned as β-orientation. Further analysis of ROESY data also showed the correlations of H-2″a/H_3-14″, and of H-7″/H-8″/H-10″, which indicated that H-2″ and H_3-14″ were assigned as β-orientation, while H-7″ and H-8″ were assigned as α-orientation (Fig. 3).

A series of sesquiterpene lactone dimers have been isolated from the genus *Inula* (Chen et al., 2016; Jin et al., 2006; Qin et al., 2009, 2010, 2011, 2012), and most of them were formed by endo/exo-selective Diels-Alder cycloaddition of two monomers with the linkage mode of either C-11/C-3′ or C-11/C-1′. Comparison of the ^{13}C NMR shift (in $CDCl_3$) of the carbonyl at C-12 [endo- (δ_C 178 ± 2) or exo-type (δ_C 184 ± 2)] with those of reported Diels-Alder cycloaddition products clearly indicated the endo-configuration of units A and B (C-12; δ_C