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Four new sesquiterpenoids from *Dendranthema morifolium* (Ramat.) kitam flowers

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ABSTRACT

Four new sesquiterpenoids, chrysanthguaianolactones C-F (1–4), together with four known sesquiterpenoids (5–8), including 3α,4α,10β-trihydroxy- 8α-acetoxyguai-1,11(13)-dien-6α,12-olide (5), 3α,4α,10β-trihydroxy-8α-acetoxy −11βH-guai-1-en-12,6α-olide (6), 8α-(angelyloxy)-3β,4β-dihydroxy-5αH,6βH,7αH, 11αH-guai-1(10)-en-12,6-olide (7) and 3β,4α-dihydroxy-8α-angelyloxy-1(10),11(13) −dien-6α,12-olide (8) were obtained from an ethyl acetate fraction, which was yielded from an acetone extract of *Dendranthema morifolium* (Ramat.) kitam. Their structures were determined with extensive spectroscopic (UV, IR, HR-ESI–MS, and 1D and 2D NMR) analyses. In addition, compounds 1–8 were evaluated for their anti-inflammatory effects on H9c2 cardiocytes impaired by lipopolysaccharide (LPS). Among them, compound 2 and compounds 5–8 exhibited anti-inflammatory effects against LPS-induced inflammation.

1. Introduction

Dendranthema morifolium (Ramat.) kitam (Flos chrysanthemum) is a traditional Chinese medicine that, has been widely used as a daily beverage for thousands of years. It was recorded in the Chinese medical classics 'Shennong's Herba' and is thought to be a 'top-grade' herb in China. Pharmacological investigations have shown that Flos chrysanthemum exhibits antibacterial (He et al., 2013), antioxidant (Lin et al., 2010), anti-inflammatory, and heart-protective (Lii et al., 2010) characteristics. Previous phytochemical studies on caffeic acid derivatives, flavonoids, triterpenoids, glycosides and alkaloids have been isolated from Flos chrysanthemum (Yuan et al., 2015; Qu et al., 2017). Based on a bioassay-guided isolation, further phytochemical study was undertaken to investigate the chemical constituents of a 50% acetone extract from Flos chrysanthemum, which led to the isolation of four new sesquiterpenoids, chrysanthguaianolactones C-F (1–4), along with four known sesquiterpenoids (5–8), 3α,4α,10β-trihydroxy-8α-acetoxyguai-1,11(13)- dien-6α,12-olide (**5**), 3α,4α,10β-trihydroxy-8α-acetoxy-11βH-guai-1-en-6α,12-olide (6), 8α-(angelyloxy)-3β,4β-dihydroxy-5αH,6βH,7αH,11αH-guai-1(10)-en-12,6-olide (7) and 3β,4α-dihydroxy-8α-angelyloxy-1(10),11(13)-dien-6β,12-olide (8). Their structures were determined in detail through extensive spectroscopic analysis (1D and 2D NMR spectroscopy and mass spectrometry) and compared with previous reports in the literature.

2. Results and discussion

The structures of the sesquiterpenoid compounds (Fig. 1) were determined through analysis using HR-ESI–MS, 1D and 2D NMR spectroscopy, including ^{1}H–^{1}H COSY, HMBC and HSQC experiments.

Compound 1 was obtained as colorless crystals.The molecular formula was determined to be $C_{20}H_{28}O_7$ by analysis of HR-ESI–MS (*m/z* 403.1725 $[M + Na]^+$, calcd. for $C_{20}H_{28}O_7Na$, 403.1727) with seven degrees of unsaturation. The IR spectrum showed the presence of OH groups (3393 cm^{-1}), CO groups (1766, 1706 cm^{-1}) and C=C bonds (1456 cm^{-1}). The ^{1}H NMR spectrum of 1 consisted of five methyl groups at δ_H 1.51 (s, H-14), 1.48 (s, H-15), 1.18 (d, J = 6.9 Hz, H-13), 1.90 (s, H-5′) and 2.01 (d, J = 7.2 Hz, H-4′); one methylene group at δ 2.13 (dd, J = 15.2, 4.8 Hz, H-9a) and 2.00 (brd, J = 15.2 Hz, H-9b); three oxygenated methane groups at δ 5.42 (m, H-8), 4.51 (t, J = 10.8 Hz, H-6) and 4.08 (d, J = 2.6 Hz, H-3); and two olefinic protons at δ 6.19 (m, J = 7.2 Hz, H-3′) and 5.98 (t, J = 2.6 Hz, H-2). The^{13}C NMR spectrum indicated four olefinic carbon signals at δ_C 153.8 (C-1), 128.2 (C-2), 128.6 (C-2′) and 140.5 (C-3′); two carbonyl carbon signals at δ 180.5 (C-12) and 168.0 (C-1′); five O-bearing carbon signals at δ 83.4 (C-3), 82.6 (C-4), 78.2 (C-6), 73.2 (C-8) and 71.7 (C-10); five methyl carbon signals at δ 29.3 (C-14), 23.2 (C-15), 20.7 (C-5′), 16.1 (C-4′) and 15.1 (C-13); one CH_2 carbon signals at δ 46.0 (C-9); and three CH carbon signals at 59.1 (C-5), 54.6 (C-7) and 42.8 (C-11). Four

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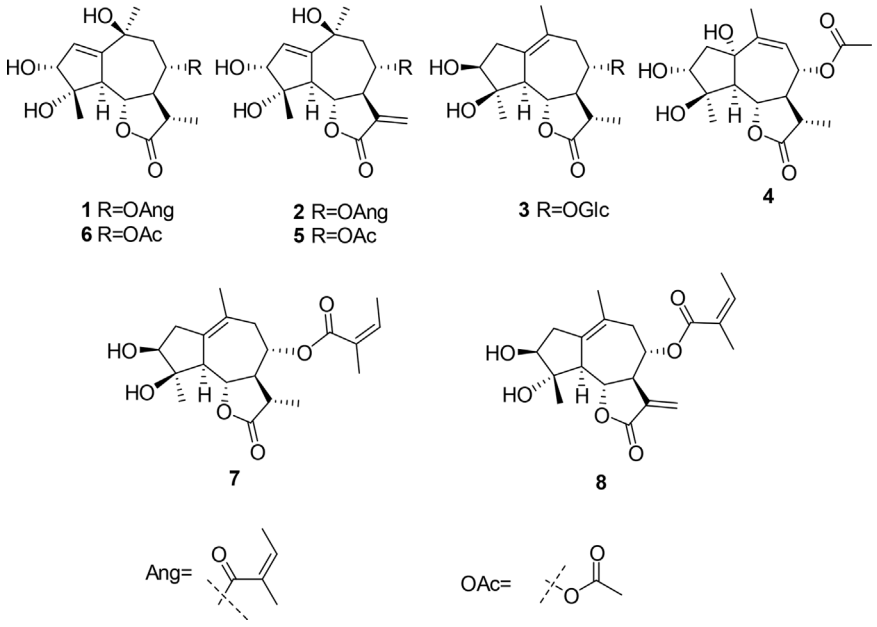

Fig. 1. Structure of compounds 1–8.

degrees of unsaturation were attributed to two C═O groups and two pairs of C═C bonds; the remaining three degrees of unsaturation indicated that 1 had a tricyclic ring skeleton. Comparison of the NMR data of 1 with indicumolide A and known sesquiterpenoids 6 (Feng et al., 2009; Tan et al., 1998), which was also isolated from this genus, suggested that 1 was a tricyclic guaiane-type sesquiterpenoid.

The ^{1}H–^{1}H COSY correlations were observed for the H-3/H-2/H-5/H-6/H-7/H-8/H-9 spin system. The planar structure of 1 was outlined mainly by HMBC experiment (Fig. 2). The HMBC spectrum clearly demonstrated correlations from Me-13 to C-7, C-11 and C-12; from Me-15 to C-4, and C-5; from Me-14 to C-1, C-9 and C-10; from H-2 to C-5, C-10, C-3 and C-1; from H-6 to C-11, C-7, C-5, C-4, C-1and C-8; from H-3 to C-5, C-4, C-2 and C-1; from H-5 to C-6, C-7, C-2 and C-1; and other correlations shown in Fig. 2. A hydroxyl group was further determined to be located at C-3 in 1 by the detailed analysis of HMBC correlations between H-2 and C-3. The remaining hydroxyls were respectively placed on C-4 and C-10 judging from the HMBC correlations with the nearby proton signals shown in Fig. 2. Additionally, the HMBC correlations from Me-5′ to C-1′, C-2′ and C-3′; from Me-4′ to C-1′, C-2′, and C-3′; and from H-3′ to C-1′, C-4′, and C-5′ revealed the presence of a 2′-methylbut-2′-enoyl moiety in **1**, and its (Z)-configuration was determined by the correlation of H-5′ with H-3′ in the NOESY experiment. Moreover, the HMBC correlation from H-8 to C-1′ indicated that the 2′-methylbut-2′-enoyl moiety was attached to C-8.

The relative configuration of 1 was deduced from the NOESY spectrum. The NOESY correlations were observed between H-2 and H-3, Me-14, between Me-15 and H-3, H-6, and between H-7 and Me-13. since the H-5 of the guaiane-type sesquiterpenoid was defined in the α-configuration (Tan et al., 1998), the structure of 1 was unambiguously elucidated as 3α,4α,10β-trihydroxy-8α-angelyloxy-11βH-guai-1-en-6α,12-olide, named chrysanthguaianolactone C.

Compound **2** was obtained as colorless crystals. The molecular formula was determined to be $C_{20}H_{26}O_7$ by analysis of HR-ESI–MS (*m/z* 401.1567 [M + Na]$^+$, calcd. for $C_{20}H_{26}O_7Na$, 401.1570) with eight degrees of unsaturation. The IR spectrum showed the presence of OH groups (3416 cm^{-1}), CO groups (1772, 1700 cm^{-1}) and C═C bonds (1456 cm^{-1}). The NMR spectroscopic data from **2** (Table 1) resembled those of 1 except for the missing resonances assigned to −CH−CH$_3$ in 1, showing an −C═CH group instead, which showed the resonance signals at C-11 (*δ* 140.4) and C-13 (*δ* 120.7). the HMBC correlations are shown in Fig. 2. Furthermore, the stereochemistry was established by a NOESY experiment, in which H-6/H-15 and H-3/H-15 correlated with each other. This established a *β*-orientation for the protons at C-3, C-6, and C-15. Additionally, H-5/H-14 showed an NOESY correlation confirming the α-orientation of the protons at C-5 and C-14. Thus, compound **2** was elucidated as 3α,4α,10*β*-trihydroxy-8α-angelyloxyguai-1,11(13)-dien-6α,12-olide, and named chrysanthguaianolactone D.

Compound **3** was obtained as colorless crystals.The molecular formula was determined to be $C_{21}H_{23}O_{10}$ by analysis of HR-ESI–MS (*m/z* 467.1888 [M + Na]$^+$, calcd. for $C_{21}H_{23}O_{10}Na$, 467.1887) with six degrees of unsaturation. The IR spectrum showed the presence of OH groups (3381 cm^{-1}), CO groups (1647 cm^{-1}) and C═C bonds (1373 cm^{-1}). The NMR spectroscopic data of **3** (Table 1) resembled those of 7 except for the missing resonances assigned to a 2′-methylbut-2′-enoyl moiety in 1, showing an a glucopyranoside group instead, which showed the resonance signals at C-1′ (*δ* 105.4), C-2′ (*δ* 75.5), C-3′ (*δ* 78.7), C-4′ (*δ* 71.5), C-5′ (*δ* 78.0), and C-6′ (*δ* 62.8). In the acid hydrolysis of 3, D-glucose was respectively afforded and confirmed by TLC and optical rotations compared with the reference substances, (Zhang et al., 2008, 2016). The configuration was determined by measuring the optical rotation value and the large $^{3}J_{H1,H2}$ coupling constant. Based on the results described above, the structure of the carbohydrate fragment of 3 was determined to be (1 → 6)-β-D-glucopyranoside. Moreover, the HMBC correlation from H-8 to C-1′ indicated

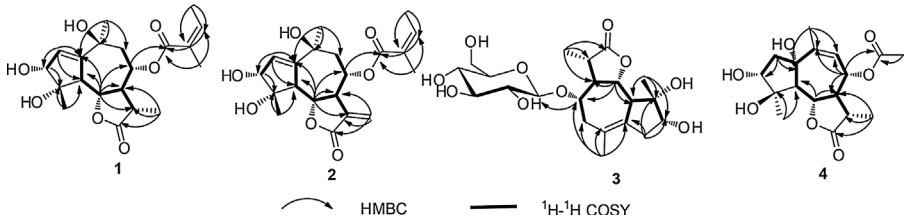

Fig. 2. Key HMBC and ^{1}H–^{1}H COSY correlations of 1–4.