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Review

Three new sesquiterpenes from *Tithonia diversifolia* and their anti-hyperglycemic activity

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ABSTRACT

Three new germacrane sesquiterpenes (**1**), (**2**), (**3**), along with eleven known sesquiterpenes, namely, tirotundin-3-*O*-methyl ether (**4**), deacetylvguiestin (**5**), 1β-hydroxydiversifolin-3-*O*-methyl ether (**6**), tagitinin C (**7**), 1β-hydroxytirotundin-3-*O*-methyl ether (**8**), 1β-hydroxytirotundin-1,3-*O*-dimethyl ether (**9**), tagitinin F-3-*O*-methyl ether (**10**), tagitinin F (**11**), tagitinin A (**12**), 3β-acetoxy-4α-hydroxyeduesm-11(13)-en-12-oic acid (**13**) and ilicic acid (**14**) were isolated from the aerial parts of *Tithonia diversifolia*. Their structures were established by spectroscopic analysis, while the relative configuration of compound **1** was confirmed by X-ray diffraction analysis. In addition, compounds **1**–**14** were evaluated *in vitro* for their anti-hyperglycemic activity by glucose uptake in 3T3-L1 adipocytes. It was found that 10 μg/mL **1**, **3**, **6** and **8** could significantly increase glucose uptake without significant toxic effects.

Contents

1. Introduction

Tithonia diversifolia (Hemsl.) A. Gray is an impressive member of the sunflower family, Asteraceae. It is a perennial native of Mexico and Central America and was introduced to the southern part of Asia, Africa and the Pacific region [1]

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because of ornamental purposes or pharmacological action. *T. diversifolia* was brought to Yunnan province of China in the 1900s. Traditionally, *T. diversifolia* is used for the treatment of malaria and other forms of fever or wound in Mexico. Presently, *T. diversifolia* is of particular interest in health care phytomedical research with respect to its antimalarial [2,3], antidiabetic [4–6], anti-inflammatory [7–10], and anticancer [11,12] activities. Chemical studies on this species have resulted in sesquiterpene lactones, chromene, and flavone [13–16]. The present study is our ongoing research of antidiabetic active metabolites from *T. diversifolia*, involving the isolation and identification of 14 sesquiterpenes, including three new sesquiterpenes: tagitinin G (1), tagitinin H (2), tagitinin I (3), and 11 known sesquiterpenes: tirotundin 3-O-methyl ether (4) [15], deacetylvguiestin (5) [16], 1β-hydroxydiversifolin-3-O-methyl ether (6) [17], tagitinin C (7) [16], 1β-hydroxytirotundin 3-O-methyl ether (8) [12], 1β-hydroxytirotundin-1,3-O-dimethyl ether (9) [18], tagitinin F-3-O-methyl ether (10) [17], tagitinin F (11) [19], tagitinin A (12) [16], 3β-acetoxy-4α-hydroxyeduesm-11(13)-en-12-oic acid (13) [20], ilicic acid (14) [21,22]. In addition, the anti-hyperglycemic activities of compounds 1-14 were evaluated by glucose uptake in 3T3-L1 adipocytes. The purity of the compounds ranged from 90.5% (14) to 97.3% (1) as determined by analytical HPLC with DAD and ELSD detection.

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR date for compounds **1, 2** and **3.**

No.	1[a]		2[a]		3[a]	
	δ_H mult (J in Hz)	δ_C	δ_H mult (J in Hz)	δ_C	δ_H mult (J in Hz)	δ_C
1α	4.00 dd (9.0, 9.0)	78.4	1.96 m	38.1	1.72 m	36.6
1β			1.78 m		1.73 m	
2α	2.22 dd (12.6, 9.0)	44.3	1.76 m	33.5	2.39 m	24.6
2β	1.63 dd (12.6, 9.0)		1.70 m		2.39 m	
3		110.4		110.3		150.1
4	1.98 dq (6.6, 19.2)	35.8	1.96 m	35.8		106.6
5α	2.24 ddd (2.4, 5.4, 7.8)	38.3	2.20 m	38.6	2.84 dd (10.8, 13.2)	39.5
5β	1.66 ddd (5.4, 12.0, 18.6)		1.68 m		2.14 d (13.2)	
6	4.40 ddd (2.4, 10.2, 12.6)	79.4	4.32 ddd (3.0, 10.8, 12.0)	80.0	4.31 dd (5.4, 10.8)	78.4
7	2.50 ddd (7.8, 10.2, 18.0)	60.3	2.47 ddd (7.8, 10.2, 18.0)	60.1	3.97 dddd (2.4, 3.0, 3.6, 5.4)	49.5
8	3.81 dd (8.4, 7.8)	69.8	3.75 dd (8.4, 7.8)	69.5	5.43 ddd (2.4, 5.4, 10.8)	70.2
9α	2.91 dd (8.4, 13.8)	38.6	2.22 m	47.2	1.64 dd (5.4, 13.8)	37.4
9β	1.60 d (8.4)		1.79 m		1.71 dd (10.8, 13.8)	
10		82.7		78.5		80.1
11	2.37 dq (6.6, 19.2)	43.0	2.32 dq (6.6, 13.2)	43.0		136.9
12		178.4		178.6		169.4
13α	1.23 d (6.6)	13.6	1.18 d (6.6)	13.9	6.27 d (3.6)	122.0
13β					5.56 d (3.0)	
14	1.40 s	26.8	1.39 s	28.8	1.47 s	23.2
15	0.95 d (7.2)	15.6	0.97 d (7.2)	15.9	1.69 s	18.5
1'						176.3
2'					2.40 m	34.0
3'					1.02 d (6.6)	19.1
4'					1.04 d (6.6)	18.7

[a] Spectra were measured in $CDCl_3$.

2. Experimental

2.1. General experimental procedures

Optical rotation was measured on a Perkin Elmer polarimeter (serial No. 9903). ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) spectra and all 2D NMR spectra were obtained on a Bruker Avance 600 NMR spectrometer (Bruker Co., Germany). Chemical shifts are expressed in δ (ppm) referring to the solvent peaks δ_H 7.26 and δ_C 77.0 for $CDC_{l3,}$ δ_H 7.22 and δ_C 135.5 for pyridine, and the coupling constant (*J*) is given in Hz. HRESI-MS was recorded on a Varian MAT-212 mass spectrometer and a Agilent Technologies 6538 UHD Accurate-Mass Q-TOF LC/MS spectrometer (Agilent Technologies, MA, USA); GC/MS(Termo Finnigan Trace GC apparatus, L-Chirasil-Val column, 25 m × 0.32 mm). IR was recorded on a Bruker Vector 22 spectrometer with KBr pellet. The purity (%) of compounds were tested with an Agilent 1200 instrument using using a diamonsil C18 column (250 mm × 4.6 mm, 5 μm) eluting with MeOH–H_2O (30%–60%) at 1.0 mL/min detected by SEDEX 75 ELSD (Dikma Technologies, USA) and DAD detector (Agilent Technologies, MA, USA). Crystallographic data were collected on a Bruker SMART diffractometer using a graphitedater with Mo Kα radiation. Other materials included Sephadex LH-20 (Pharmacia), Silica gel GF254 (Luyou company of Yantai, 100–200 and 200–300 mesh); RP-C18 (Merck, 43–60 μm), TLC GF254 and preparative TLC GF254 (Luyou company of Yantai); TLC Silica gel 60 RP-18F254 (Merck); MCI gel (Mitsubishi chemical corporation); 3T3-L1 cell line (Tsucuba Cell Bank, Japan); Pioglitazone (Sigma Aldrich, P4120; purity ≥ 99.9%) was used as a positive control.

2.2. Plant material

The aerial parts of *T. diversifolia* (Hemsl.) A. Gray were collected in Mengzi of Yunnan province, China in September 2007 and identified by Prof. Wansheng Chen (Department of Pharmacy, Changzheng Hospital, Second Military Medical University). A voucher specimen (no. 20070820) was deposited in the Department of Pharmacognosy of the Second Military Medical University in Shanghai, China.

2.3. Extraction and isolation

The dried aerial parts of *T. diversifolia* (21 kg) were percolated with 80% EtOH at room temperature. The EtOH extract was concentrated to an aqueous residue and suspended with water. The water layer was extracted with petroleum ether, EtOAc and n-BuOH, and the EtOAc soluble fraction (128.0 g) was separated by column chromatography using silica gel with Petroleum ether-EtOAc (30:1; 15:1; 8:1; 4:1; 2:1; 1:1), resulting in six major fractions (1–6). Fraction 2 (6.5 g) was further separated