ORIGINAL ARTICLE

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Rec. Nat. Prod. X:X (202X) XX-XX

records of natural products

Tigliane-Type Diterpenoids from the Seeds of *Croton tiglium*

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(Received May 07, 2022; Revised July 17, 2022; Accepted July 19, 2022)

Abstract: A new tigliane-type diterpenoid (**1**) and three known analogues (**2**–**4**) were isolated from the seeds of *Croton tiglium*. Extensive spectroscopic analyses, especially the 2D NMR experiments were used to determine their structures. The absolute configuration of **1** was defined by single-crystal X-ray diffraction data. The cytotoxicity **1**–**4** was evaluated against melanoma cell line A375, and the results showed that compounds **1**, **3**, and **4** exhibited certain cytotoxicities.

Keywords: *Croton tiglium*; tigliane-type diterpenoid; cytotoxicity.

1. Introduction

Tigliane diterpenoids are a class of diterpenoids with a 5/7/6/3-fused carbon skeleton. Most of this type compounds were mainly found in Euphorbiaceae and Thymelaeaceae plants, and were reported to possess a wide range of biological properties [1]. *Croton tiglium* L. belongs to Euphorbiaceae family, and the seeds of this plant are well known as “Ba Dou” in traditional Chinese medicine to treat evil sores and scabies [2]. Previous studies have shown that tigliane diterpenoids were the main components in this plant, and many of these compounds exhibit diverse bioactivities, including cytotoxic, antiviral and anti-inflammatory activities [3–8]. In the present study, a new tigliane diterpenoid (**1**) and three known analogues (**2**–**4**) were obtained from the seeds of *C. tiglium* (Figure 1). The structures were characterized by spectroscopic data analyses. Herein, the isolation and structural identification of compounds **1**–**4**, as well as their cytotoxicites are reported.

2. Materials and Methods

2.1. General Experimental Procedures

X-ray data were obtained via a Bruker APEX-II CCD diffractometer, and the melting point was measured by an X-4 melting instrument. UV spectrum were obtained using Shimadzu UV-2450

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 202x EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.344.2205.2455
Available online: July 29, 2022

spectrophotometry. Optical rotation data were obtained by a Rudolph Autopol I automatic polarimeter, and IR data were obtained using a Bruker Tensor 27 spectrometer. NMR data were obtained using a Bruker AM-500 spectrometer. HRESIMS and HPLC were performed on Agilent Q-TOF micro mass spectrometer and Agilent 1200 series, respectively. Macroporous resin D101 (Donghong Chemical Co., Ltd.) was used for column chromatography. TLC analysis was performed on silica gel plates (Marine Chemical Ltd.).

2.2. Plant Materials

The seeds of *C. tiglium* were collected in September 2020, from Guangxi Province, P.R. China, and were identified by Professor Jianyong Zhu. A voucher specimen (CT202009) was deposited at our laboratory.

2.3. Extraction and Isolation

The seeds of *C. tiglium* (2.5 kg) were pulverized and extracted with 90% ethanol (25 L), each 7 days at rt. The solvent was evaporated in vacuum to give an extract (395.0 g), which was suspended in water and separated with EtOAc. The EtOAc extract (312.0 g) was separated over macroporous resin column, eluting with MeOH-H_2O (5:5 → 9:1, v/v), to yield five fractions (Fr.A–Fr.E). Fr.C (42.2 g) was separated by CC on silica gel eluted with (petroleum ether-EtOAc, 15:1 → 1:2, v/v) afford Fr.C1–Fr.C5. Fr.C3 (1.2g) was separated over Sephadex column, and then purified via HPLC (MeOH-H_2O, 8:2, v/v) to give **3** (12.5 mg) and **4** (58.0 mg). Compound **1** (4.2 mg) and **2** (31.8 mg) were isolated from Fr.C4 (300.0 mg) in a similar way.

2.4. Spectral Data

*Compound **1**:* colorless crystals, mp 166–168 °C; $[\alpha]^{25}_{D}$ −16.8 (*c* 0.25, MeCN); UV (MeCN) λ_{max} (log ε) 223 (3.26), 195 (3.27) nm; IR (KBr) ν_{max} 3405, 2927, 1693, 1376, 1261, 1248, 734 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 411.1782 $[M + Na]^+$ (calcd for $C_{22}H_{28}O_6Na^+$, 411.1778).

*2.5. X-ray Crystallographic Data of **1***

$C_{22}H_{28}O_6$ (*M* = 388.44 g/mol): orthorhombic, space group $P2_12_12_1$ (no. 19), *a* = 8.88430(10) Å, *b* = 9.46280(10) Å, *c* = 24.2964(3) Å, *V* = 2042.61(4) $Å^3$, *Z* = 4, *T* = 100.00(10) K, μ(Cu Kα) = 0.748 mm^{-1}, *Dcalc* = 1.263 g/cm^3, 20960 reflections measured (7.276° ≤ 2Θ ≤ 157.75°), 4323 unique (R_{int} = 0.0383, R_{sigma} = 0.0247) which were used in all calculations. The final R_1 was 0.0312 (I > 2σ(I)) and wR_2 was 0.0824 (all data). Flack parameter = −0.01(6). The complete data were deposited at the Cambridge Crystallographic Data Centre (CCDC 2170690).

2.6. Cytotoxicity Assay

A375 human malignant melanoma cells were obtained from the cell bank of Chinese Academy of Sciences (Shanghai, China) and were cultivated in DMEM containing 10% FBS and 0.5% penicillin/streptomycin. The cells were incubated with 5% CO_2 at 37 °C in a humidified environment. The cytotoxicity for compounds **1**–**4** was measured by MTT method as our previously described [9].

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was obtained as colourless block crystals and assigned a molecular formula of $C_{22}H_{28}O_6$ by the HRESIMS positive peak at *m*/z 411.1782 $[M + Na]^+$ (calcd for $C_{22}H_{28}O_6Na^+$,

411.1778). The IR data of **1** showed absorptions at 3405 cm^{-1} (hydroxyl group) and 1693 cm^{-1} (carbonyl group). The ^{1}H NMR spectrum (Table 1) of **1** displayed one aldehyde group (δ_H 9.30, 1H, s, H-20); two olefinic protons at δ_H 7.01 (1H, br s, H-1) and 6.08 (1H, t, J = 2.9, H-7); one oxygenated methine (δ_H 4.05, 1H, d, J = 9.7, H-12); and five methyl groups at δ_H 1.25 (3H, s, Me-16), 1.29 (3H, s, Me-17), 1.29 (3H, d, J = 6.3 Me-18), 1.75 (3H, s, Me-19) and 2.10 (3H, s, Me-2′). The ^{13}C NMR spectrum of **1** showed 22 carbons comprising a ketocarbonyl group (δ_C 210.3), an aldehyde group (δ_C 194.2), a carbonyl group (δ_C 173.8), two double bonds (δ_C 155.0, 152.7, 143.6 and 140.3), a quaternary carbon, two oxygenated tertiary carbons, five methyls, six sp^3 methines (one oxygenated), and a sp^3 methylenes. The aforementioned spectroscopic data implied that compound **1** was a tigliane diterpenoid with an acetyl group [8].

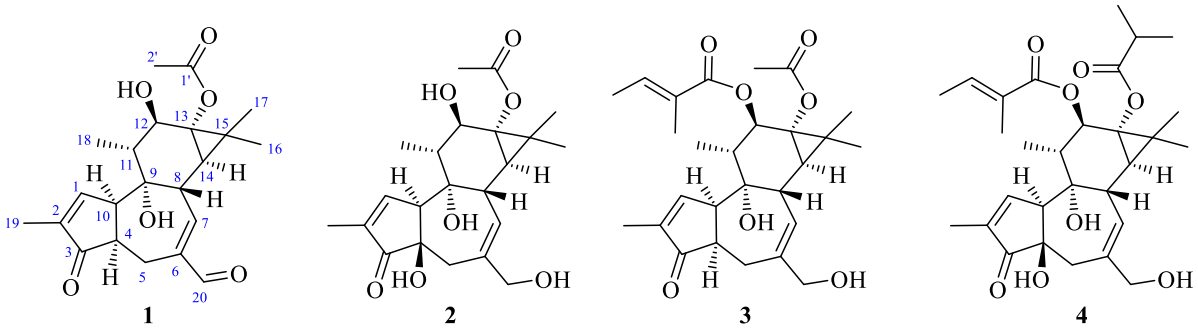

Figure 1. Structures of compounds **1**–**4** from *Croton tiglium*

In the ^{1}H–^{1}H COSY spectrum, three fragments (C-1–C-10–C-4–C-5, C-7–C-8–C-14 and C-12–C-11–C-18) were established. The above fragments combined with HMBC correlations of Me-19/C-1, C-2 and C-3; H-1 and H-4/C-3; H-20/C-5, C-6 and C-7; H-7 and H-10/C-9; Me-18/C-9, C-11 and C-12; H-14/C-12 and C-13; Me-16 and Me-17/C-13, C-14 and C-15 indicated the existence of a typical 5/7/6/3-fused tigliane skeleton. The weak HMBC correlation from Me-2′ to C-13 confirmed the location of the acetoxyl group at C-13. Thus, the planar structure of **1** was defined as shown in Figure 2.

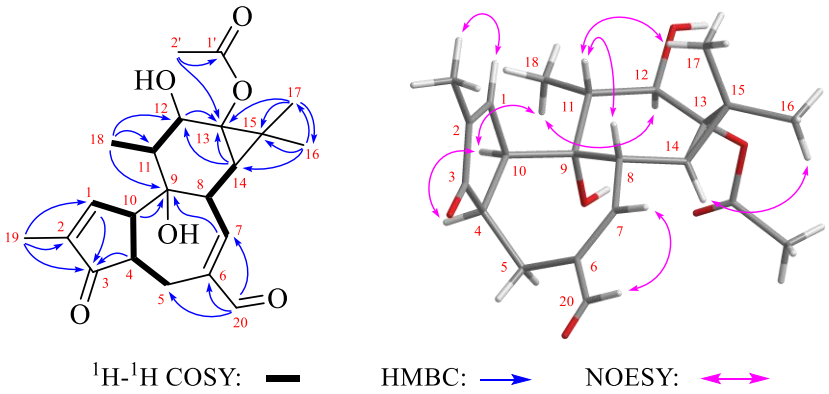

Figure 2. Key HMBC, ^{1}H–^{1}H COSY and NOESY correlations for compound **1**

The relative configuration of **1** was assigned by the NOESY spectrum. The NOE interactions (Figure 2) of H-11/H-8 and Me-17 indicated that they were cofacial and defined to be β-orientation. Thus, H-4/H-10, H-10/Me-18, Me-18/H-12 and H-14/Me-16 revealed that H-4, H-10, H-12 and H-14 were α-orientations. In addition, the double bond between C-1 and C-2 was deduced as a *Z*-configuration by NOE interaction of H-1/Me-19, while the double bond between C-6 and C-7 was determined as *E*-configuration based on NOE correlation of H-7/H-20. Finally, it is absolute

configuration of 4*R*,8*S*,9*R*,10*R*,11*R*,12*R*,13*S*,14*R* (Figure 3) was confirmed by Cu Kα single-crystal X-ray diffraction with the Flack parameter of −0.01(6). Thus, the structure of **1** was assigned as 4-deoxy-20-oxophorbol-13-acetate.

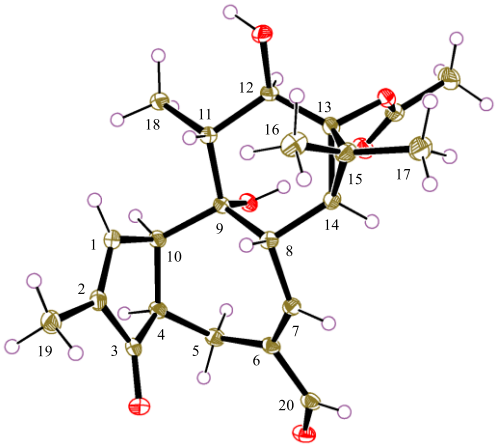

Figure 3. X-ray crystallographic structure for compound **1**

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data for compound **1** in $CDCl_3$

Position	δ_H (*J* in Hz; δ in ppm)	δ_C	Position	δ_H (*J* in Hz; δ in ppm)	δ_C
1	7.01, br s	155.0	12	4.05, d (9.7)	74.9
2		143.6	13		67.4
3		210.3	14	0.87, d (5.5)	34.9
4	2.84, m	48.4	15		26.0
5α	3.05, ddd (15.9, 6.4, 2.9)	20.7	16	1.25, s	24.0
5β	3.27, dd (15.9, 4.4)		17	1.29, s	16.4
6		140.3	18	1.29, d (6.3)	12.3
7	6.08, t (2.9)	152.7	19	1.75, s	10.5
8	2.25, m	41.6	20	9.30, s	194.2
9		77.7	1′		173.8
10	3.54, m	47.1	2′	2.10, s	21.1
11	1.64, m	44.9			

The known compounds, phorbol-13-acetate (**2**) [10], 12-*O*-tiglyl-4α-deoxyphorbol-13-acetate (**3**) [11] and 12-*O*-tiglylphorbol-13-isobutyrate (**4**) [12] were identified via comparing their data with literature values.

3.2. Cytotoxic Activity

All compounds (**1**–**4**) were screened for cytotoxicities against A375 cells using the MTT assay, and dacarbazine was used as a positive drug (IC_{50} = 23.5 ± 2.8 μM). The results displayed that compounds **1**, **3** and **4** exhibited moderate cytotoxic activities with IC_{50} values of 32.6 ± 3.5, 18.5 ± 1.8 μM and 26.4 ± 2.2 μM, respectively, while **2** had no obvious cytotoxicity (IC_{50} > 50 μM).

Acknowledgments

This work was supported by the Anhui Province Education Department Nature and Science Fund Key Project of China, Hefei, China (No. KJ2018ZD065) and the Special Fund for Modern Chinese

medicine technology innovation and social service team of Bozhou Vocational and Technical College (Nos. yptd001 and ypzk002).

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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