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Triptergosidols A-D, nerolidol-type sesquiterpene glucosides from the leaves of *Tripterygium wilfordii*

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ABSTRACT

Four new nerolidol-type sesquiterpene glucosides, triptergosidols A-D (**1**-**4**) were isolated from the leaves of *Tripterygium wilfordii*. Three aglycones, named triptergerols A (**1**a), B (**2**a), and C (**3**a), were acquired by enzymatic hydrolysis of **1**-**3**. The structures of nerolidol-type sesquiterpenes were elucidated on base of kinds of spectroscopic analysis, and their absolute configurations were determined by CD method. In addition, compounds **1**-**4** were tested for cytotoxicity against two cell lines and inhibitory effects against NO production in RAW264.7 macrophage.

1. Introduction

Tripterygium wilfordii Hook.f. (TWHF) is one of species in the genus *tripterygium*, which has been used widely in traditional medicine [1], for treatment of inflammation [2], psoriasis, systemic lupus erythematosus, ankylosing spondylitis, and idiopathic IgA nephropathycancer in China [3]. TWHF has attracted great attention for natural product research during the last five decades, which have contributed more than 200 new compounds so far, including alkaloids [4,5], triterpenoids [6], diterpenes [7], sesquiterpenes [8], megastigmane glycosides [9], flavanones [10] and lignans [11]. We have been working on the significant bioactivities leaves of TWHF, and four new nerolidol-type sesquiterpene glucosides (Fig. 1) were acquired. Notably, their aglycons, triptergerol A-C (**1**a-**3**a, Fig. 1), which were liberated by Hydrolysis of **1**-**3** with snailase, were also new compounds. Herein we report the isolation, structure elucidation, and biological activities of them.

2. Results and discussion

Triptergosidol A (**1**) was obtained as colorless oil with a molecular formula of $C_{21}H_{36}O_8$, based on HRESIMS analysis (*m/z* 439.2316 $[M + Na]^+$, calcd for 439.2302). The molecular formula accounted for four indices of hydrogen deficiency. The IR spectrum of **1** showed absorption bands at 3380 and 1645 cm^{-1} ascribable to hydroxyl and double bond functions, respectively. The ^1H and ^{13}C NMR (Table 1) spectra of **1** showed signals assignable to 1 *β*-glucose [δ_H 4.38 (1H, d, J = 6.8 Hz, H-1′); δ_C 103.9 (C-1′), 75.3 (C-2′), 78.1 (C-3′), 71.6 (C-4′), 77.9 (C-5′), 62.7 (C-6′)] and 15 carbons of aglycone moiety. The aglycone contained four tertiary methyls [δ_H 1.78, 1.24, 1.22, 1.20 (3H each, all s, H-12, 13, 14, and 15)], a vinyl ABX system at δ_H 5.04 (1H, br d, J = 10.8 Hz, H-1a), 5.21 (1H, dd, J = 17.4, 1.5 Hz, H-1b), 5.96 (1H, dd, J = 17.4, 10.8 Hz, H-2), a *trans*-double bond at δ_H 5.63 (1H, m, H-5) and 6.14 (1H, d, J = 15.5 Hz, H-6), a olefin proton at δ_H 5.71 (1H, m, H-8), one oxygenated methine at δ_H 3.64 (1H, m, H-10), together with two methylenes, and two quaternary carbons. Compound **1** was deduced to be a noncyclic sesquiterpene glycoside to meet the requirement of unsaturation degrees on the basis of the above analysis. As shown in Fig. 2, the ^1H–^1H COSY experiment on **1** indicated the presence of a partial structure, written in bold lines, and in the HMBC experiment (Fig. 2), long range correlations were observed between the following: H_3-12 and C-10 (δ_C 87.8), C-11 (δ_C 73.6); H-6 and C-4 (δ_C 47.0), C-8 (δ_C 129.3); H_3-14 and C-6 (δ_C 139.1), C-7 (δ_C 135.5), C-8; H_3-15 and C-2 (δ_C 146.4), C-3 (δ_C 73.9), C-4. HMBC correlations from H-10 (δ_H 3.64, m) to C-1′ (δ_C 103.9); from H-1′ [δ_H 4.38 (1H, d, J = 6.8 Hz] to C-10 (δ_C 87.8) suggested that the glucose was connected to C-10.

The relative stereostructure of **1** was characterized by the NOE measurement and coupling constant (Fig. 3). Irradiation of H_3-14 enhanced the signal at H-5, and H-6 enhanced the signal H-8, and no NOE was observed between H-8 and H_3-14. Thus, it was proven that H-5 and H_3-14 were on the same side, and H-6 and H-8 was on the other side. The relative configuration of double bonds between C-5 and C-6 was deduced as *E* by the coupling constant [δ_H 6.14 (1H, d, J = 15.5 Hz, H-6)].

Hydrolysis of **1** with snailase gained its aglycone (**1a**) and glucose.

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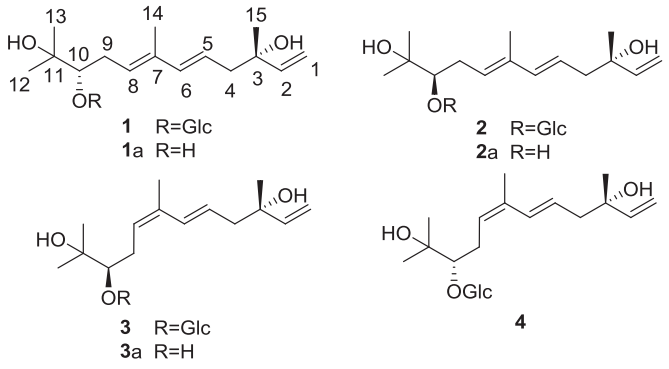

Fig. 1. The structures of compounds **1-4** and **1**a-**3**a.

Table 1
^{1}H and ^{13}C NMR spectrosopic data assignments for **1** and **1**a.

NO.	**1**[a]		**1**a[b]	
1a	5.04 (d, 10.8)	112.0	5.03 (dd,10.8, 1.5)	112.0
1b	5.21 (d,17.4)		5.20 (dd,17.4, 10.8)	
2	5.96 (dd, 17.4, 10.8)	146.4	5.95 (dd,17.4, 10.8)	146.4
3		73.9		73.9
4	2.32 (m)	47.0	2.31 (m)	47.0
5	5.63 (m)	123.7	5.61 (m)	123.4
6	6.14 (d,15.5)	139.1	6.12 (d,15.5)	139.2
7		135.5		135.8
8	5.71 (m)	129.3	5.56 (t,7.1)	129.8
9a	2.32 (m)	31.1	2.15 (m)	31.3
9b	2.52 (m)		2.50 (m)	
10	3.64 (m)	87.8	3.40 (m)	79.8
11		73.6		73.7
12	1.20 (s)	26.3	1.21 (s)	25.9
13	1.22 (s)	25.1	1.18 (s)	24.8
14	1.78 (s)	12.8	1.76 (s)	12.9
15	1.24 (s)	27.2	1.24 (s)	27.1
1′	4.38 (d,6.8)	103.9		
2′	3.23 (m)	75.3		
3′	3.23 (m)	78.1		
4′	3.31 (m)	71.6		
5′	3.38 (m)	77.9		
6′	3.66 (m),3.87 (m)	62.7		

δ in ppm; J in Hz within parentheses.
[a] Measured at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR in CD_3OD.
[b] Measured at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR in CD_3OD.

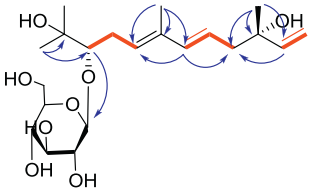

Fig. 2. Key $^{1}H-^{1}H$ COSY and HMBC correlations of **1**.

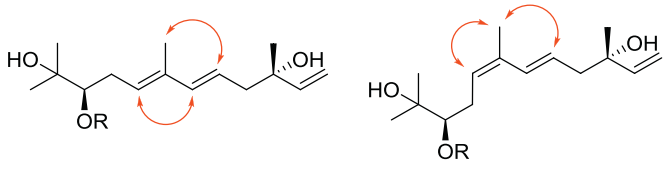

Fig. 3. Key 1D-NOE correlations of **1** and **3**.

The glucose was purified by column chromatography over silica gel and determined to D-(+)-glucose by comparison their optical rotation {D-(+)-glucose had positive optical rotation ($[\alpha]_D^{25}$ + 42.3), and the optical rotation of glucose is $[\alpha]_D^{25}$ + 48.3}. Two different methods was employing to identify the absolute configuration to the C-3 and C-10. Due to the presence of a 10,11-diol, the absolute configuration of **1** at the C-10 carbon could be ascertained through CD analysis using $Mo_2(OAc)_4$ [12,13]. The observed sign of the diagnostic band at 318 nm in the induced circular dichroism of 1-[$Mo_2(OAc)_4$] was positive (Fig. 4). According to Snatzke's method, the absolute configurations of C-10 was determined to be *S*. And the 10*S* absolute configuration showed a positive cotton effect at 230 nm in the CD spectrum. The *S* absolute configuration at the C-3 of compound **1** has deduced using double bonds of the CD helicity rule [14,15]. Since the CD spectrum of **1** showed a positive cotton effect at 200 nm, the 3-position was determined to be *S* configuration.

Therefore, the structure of **1** was identified as (3*S*,5*E*,7*E*,10*S*)-10-*β*-D -glucopyranosyloxy-3,11-dihydroxy-3,7,11-trimethyldodeca-1,5,7-triene, named triptergosidol A. Its aglycon, also was a new compound, named triptergerol A.

Triptergosidol B (**2**) was obtained as colorless oil. Its molecular formula, $C_{21}H_{36}O_8$, was the same as **1**, as determined by HR-ESI-MS. The NMR data of **2** were similar to those of **1** except C-10 (Table 2). These data suggested that **2** was an analogue of **1** eith different configuration at C-10, which was further confirmed by CD spectrum. The CD spectrum of **2** showed a negative cotton effect at 214.5 nm (Fig. 5), the 10-position was determined to be *R* configuration. Its aglycon, which was liberated by Hydrolysis of **2** with snailase(Table 3), was also a new compound, named triptergerol B (**2**a).

Triptergosidol C (**3**) as obtained as colorless oil which exhibited the molecular ion peak at *m/z* 439.2316 $[M + Na]^+$ as the base peak in the HRESIMS spectrum. Its molecular formula was determined to be $C_{21}H_{36}O_8$, the same as for **1** and **2**. A detailed comparison of NMR data revealed that **3** is differed from **2** mainly at C-7 and C-8 (Table 2). NOE correlations of H-8 and 7-CH_3 confirmed the *cis*- configurations for double bond between C-7 and C-8. Therefore, the structure of **1** was identified as (3*S*,5*E*,7*Z*,10*R*)-10-*β*-D-glucopyranosyloxy-3,11-dihydroxy-3,7,11-trimethyldodeca-1,5,7-triene, named triptergosidol C. Its aglycon, also was a new compound, named triptergerol C (Table 3).

Triptergosidol D (**4**) was obtained as colorless oil. Its molecular formula, $C_{21}H_{36}O_8$, was the same as **1**, **2**, and **3**, as determined by HR-ESI-MS. The NMR data of **4** were similar to those of **3** except C-10 (Table 2). These data suggested that **4** was an analogue of **3** with different configuration at C-10, which was further confirmed by CD spectrum. The CD spectrum of **4** showed a positive cotton effect at 240.5 nm (Fig. 5), the 10-position was determined to be *S* configuration.

Four nerolidol-type sesquiterpene glucosides were evaluted for cytotoxic activities against A549 and Hela, and no compounds showed obvious activity at a concentration of 50 μM. Compounds **1** and **2** exhibited inhibitory effects with inhibition ratio value of 65.2 ± 7.3% and 58.2 ± 5.6% at concentration 60 μM against NO production in LPS-induced RAW264.7 macrophage.

3. Experimental section

3.1. General experimental procedures

Optical rotation was performed in a JASCO P2000 automatic digital polarization device, which is from Tokyo, Japan. The CD spectra were performed on the JASCO J-815 CD spectrometer. The UV spectra were measured on JASCO v-650 spectrophotometer and IR spectra were carried out a Nicolet 5700 spectrometer (thermal electron, Madison, USA). The NMR spectra were obtained by the AV-600-III spectrometer (Bruker, USA) and INOVA-500 (Varian, palo Alto, California, USA). HRESIMS spectra were recorded an Agilent 1100 series LC/MSD ion trap mass spectrometer (Agilent, Waldbronn, Germany). HPLC was prepared using the Shimadzu LC-6AD instrument with SPD-20A detector(Shimadzu, Kyoto, Japan) and YMC -pack ODS (YMC, 250 × 20 mm, 5 μm Kyoto). Using silica gel (200–300 mesh) and polyamide

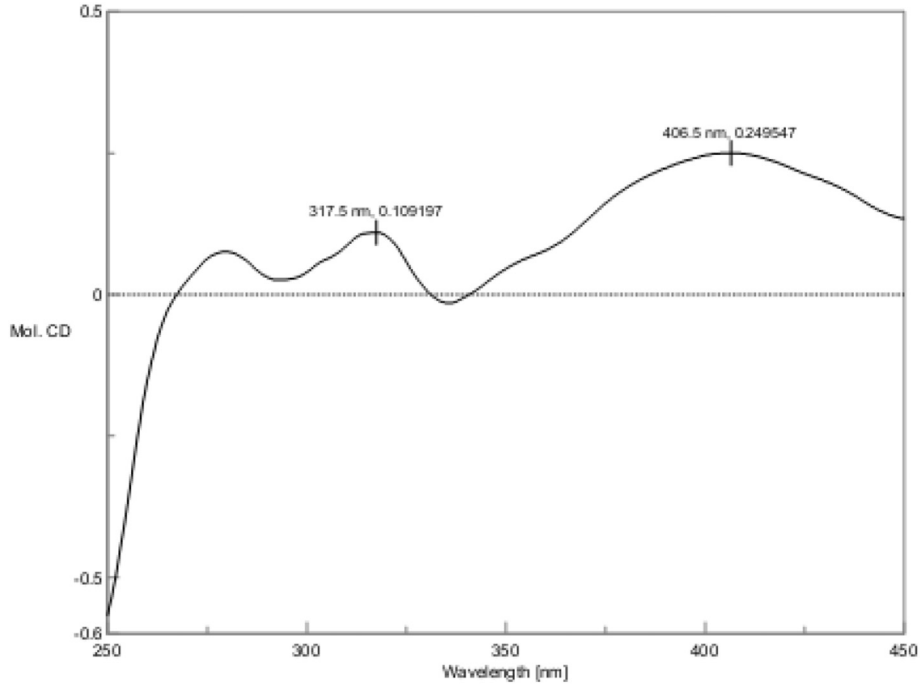

Fig. 4. CD spectrum of compound **1a** in a DMSO of dimolybdenum tetraacetate (the inherent CD of the diol was subtracted).

Table 2
^{1}H and ^{13}C NMR spectrosopic data assignments for **2-4**.

NO.	**2**		**3**		**4**	
1a	5.04 (br.d,10.8)	112.0	5.05 (dd, 10.8, 1.3)	112.1	5.05 (dd, 10.8, 1.5)	112.1
1b	5.21 (dd,17.3,1.5)		5.21 (dd, 17.4, 1.3)		5.22 (dd, 17.4, 1.5)	
2	5.96 (dd,17.4,10.8)	146.4	5.96 (dd, 17.4, 10.8)	146.3	5.97 (dd, 17.4, 10.8)	146.4
3		74		73.9		73.9
4	2.33 (m)	47	2.37 (m)	47.3	2.37 (m)	47.3
5	5.59 (td,15.6,7.5)	123.8	5.72 (dt, 15.6, 7.5)	126.6	5.77 (dt, 15.6, 7.5)	127.2
6	6.17 (d,15.6)	139.3	6.49 (d, 15.6)	131.6	6.52 (dt,15.6)	131.2
7		134.9		133.3		134.1
8	5.77 (t,7.0)	129.9	5.66 (t, 6.9)	127.8	5.59 (t,7.5)	127.3
9a	2.33 (m)	31.4	2.33 (m)	30.6	2.38 (m)	30.3
9b	2.49 (m)		2.55 (m)		2.58 (m)	
10	3.50 (m)	90.4	3.49 (dd, 8.2, 3.6)	90.6	3.60 (dd, 8.1, 3.8)	88.3
11		74.7		74.7		73.6
12	1.21 (s)	26.3	1.23 (s)	24.5	1.2 (s)	26.4
13	1.23 (s)	24.5	1.21 (s)	26.4	1.22 (s)	25
14	1.75 (s)	12.9	1.83 (s)	20.9	1.83 (s)	20.9
15	1.25 (s)	27.1	1.26 (s)	27.2	1.26 (s)	27.2
1′	4.37 (d,7.8)	106.4	4.45 (d,7.8)	106.4	4.37 (d,7.7)	104.1
2′	3.26 (m)	75.9	3.25 (m)	75.9	3.25 (m)	75.3
3′	3.35 (m)	78.3	3.36 (m)	78.3	3.36 (m)	78.1
4′	3.34 (m)	71.6	3.33 (m)	71.6	3.33 (m)	71.6
5′	3.27 (m)	77.8	3.28 (m)	77.8	3.28 (m)	77.9
6′	3.72 (m), 3.87 (m)	62.8	3.73 (m), 3.88 (m)	62.9	3.67 (m), 3.87 (m)	62.6

δ in ppm; J in Hz within parentheses; Measured at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR in CD_3OD.

(60–100 mesh) were from Qingdao Marine chemical company, Jiangsu Changfeng chemical company, D101 macroporous purchased from the Tian jin Huang hua chemical Co.LTD and need the ODS (50 μm, Tokyo, Japan). Thin layer chromatography was performed on glass coated silica gel GF254. The spots can be seen under UV light, or 10% of sulfuric acid sprayed in EtOH and then heated.

3.2. Plant material

The leaves of *T. wilfordii* were collected in Shanming, Fujian Province, People's Republic of China (PRC), in September 2009 and identified by Professor Lin Ma from the Institute of Materia Medica (IMM), Chinese Academy of Medical Sciences and Peking Union Medical College (CAMS & PUMC). A voucher specimen (No. 20090034) is deposited at the herbarium of the IMM, CAMS & PUMC, PRC.

3.3. Extraction and isolation

After drying at room temperature, the leaves of *T. wilfordii* (100 kg) were pulverized and refluxed with EtOH-water (80:20, v/v, 400 L × 2 h × 3). Solvent was evaporated *in vacuo*, and the fluid extract was diluted with water, and then extracted with EtOAc. The water portion was subjected to column chromatography (CC) over polyamide by elution with water and EtOH-water (1:1, v/v). Then the water eluant was subjected to CC over D101 macroporous adsorption resin by elution with water and EtOH-water (30%, 60%, and 95%, v/v, successively) given fractions A1-A4. Fraction A3 (338.5 g) with similar weight diatomite was in sequence to reflux with EtOAc, EtOH, and MeOH (fraction B1, B2 and B3, respectively). Fraction B2 (151.38 g) was subjected to CC over 200–300 mesh silica gel with $CHCl_3$-MeOH (15: 1–1: 1, v/v) to acquisition fractions C1 - C7. Then fraction C6 (4.373 g) was subjected to an C18 column with CH_3OH-water (20%–70%), and finally purified by preparative HPLC (detected at 230 nm, 8 mL/min) to give **1** (12 mg, CH_3CN-H_2O, 20:80, v/v, t_R: 82 min), **2** (8 mg, CH_3CN-H_2O, 20:80, v/v, t_R:86 min), **3** (8 mg, CH_3CN-H_2O, 20:80, v/v, t_R: 78 min), and **4** (3 mg, CH_3OH-H_2O, 40:60, v/v, t_R: 92 min).

3.4. Enzymatic hydrolysis of *1, 2 and 3*

Enzymatic hydrolysis of **1**, **2** and **3** followed the literature [16] with snailase: compounds **1**, **2** and **3** (8.2, 5.6 and 5.3 mg, respectively), dissolved in H_2O (1.0 mL) with snailase (10.0, 8.0 and 8.0 mg, respectively), was stirred at 37 °C for 8 h. After the reaction and cooling, the mixture was extracted with EtOAc. The water layer was subjected to CC on silica gel (300–400 mesh) with CH_3CN-H_2O (8:1, v/v) to afford

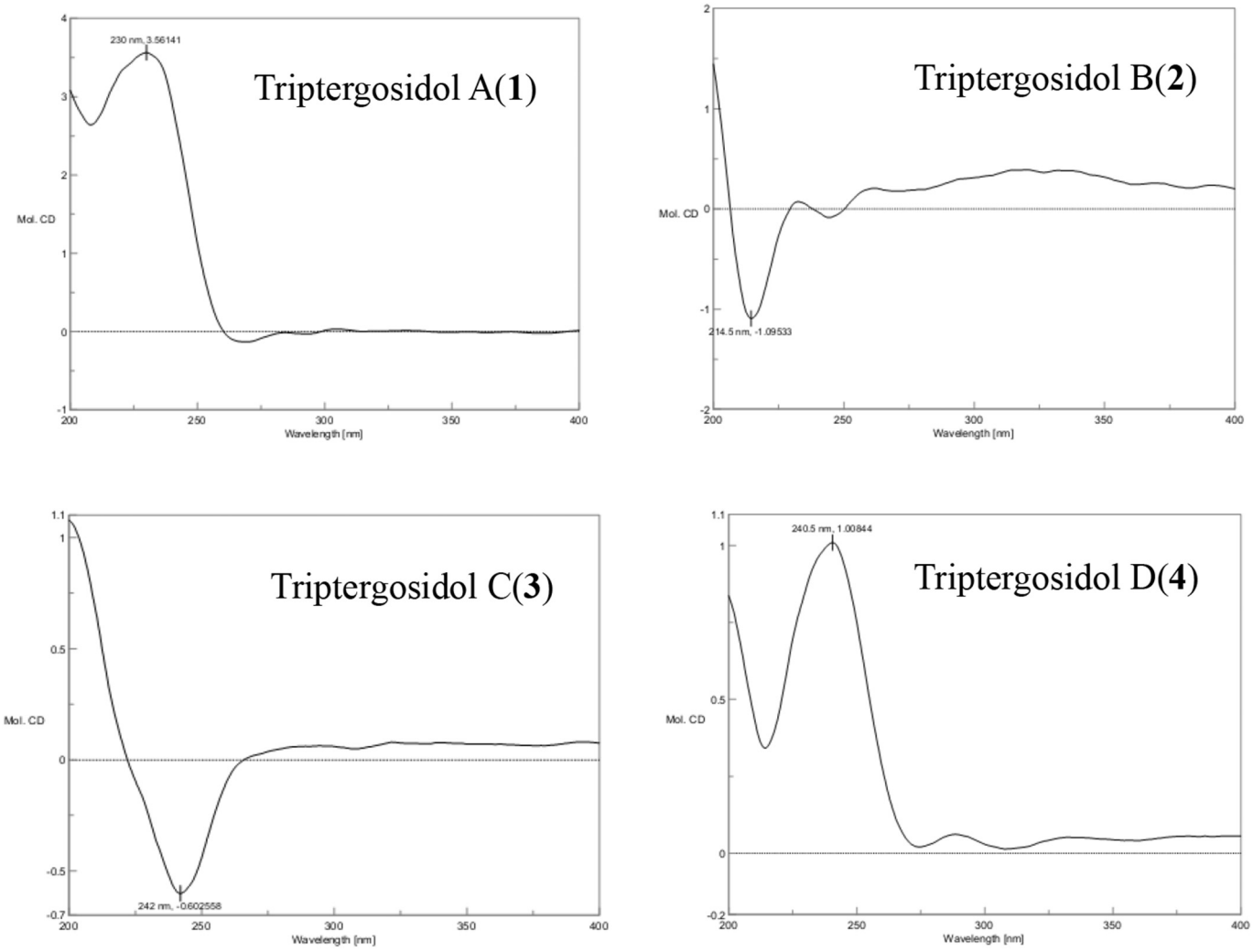

Fig. 5. The CD curve of Triptergosidols A-D (**1-4**).

Table 3
^{1}H and ^{13}C NMR spectrosopic data assignments for **2**a and **3**a.

NO.	**2**a		**3**a	
1a	5.04 (dd,10.8,1.5)	112.0	5.04 (dd,10.8,1.5)	112.1
1b	5.20 (dd,17.4,1.5)		5.21 (dd,17.4,1.5)	
2	5.95 (dd,17.4,10.8)	146.4	5.96 (dd,17.4,10.8)	146.4
3		73.7		73.8
4	2.33 (m)	47.0	2.36 (m)	47.3
5	5.61 (td,15.6,7.0)	123.4	5.73 (dt,15.6,7.5)	126.7
6	6.13 (d,15.6)	139.2	6.51 (d,15.6)	131.5
7		135.1		134.2
8	5.56 (t,7.1)	129.8	5.45 (t,7.2)	127.8
9a	2.16 (m)	31.3	2.23 (m)	30.4
9b	2.49 (m)		2.54 (m)	
10	3.34 (overlap)	79.8	3.33 (overlap)	79.9
11		73.9		73.9
12	1.18 (s)	24.8	1.18 (s)	24.8
13	1.21 (s)	25.9	1.25 (s)	25.9
14	1.76 (s)	12.9	1.84 (s)	21.0
15	1.24 (s)	27.1	1.25 (s)	27.2

δ in ppm; J in Hz within parentheses; Measured at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR in CD_3OD.

glucose (1.8 mg). The EtOAc layer was purified by HPLC (30% CH_3CN-H_2O, v/v, t_R: 78 min, detected at 230 nm, 8 mL/min) to acquired **1**a (3.3 mg), **2**a (1.2 mg) and **3**a (1.0 mg).

3.5. Determination of absolute configuration of the 10,11-diol unit in *1*

Snatzke's method was used to consider the absolute configuration of the C-10 in **1**. The detailed operation instructions were the same as published procedure [13].

3.6. Isolated new compounds

3.6.1. Triptergosidol A (1)

Colorless oil; $[\alpha]_D^{25}$ −233.6 (*c* 0.1 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 200.8 (2.77) nm, 235.6 (2.92); IR (microscope) ν_{max} 3380, 2974, 1645, 1374, 1079, 1041, 970, 924, and 641 cm^{-1}; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$) 200 (+3.10), 230 (+3.56); ^{1}H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz), see Table 1; HRESIMS *m/z* 439.2316 $[M + Na]^+$ (calcd for $C_{21}H_{36}NaO_8$, 439.2302).

3.6.2. Triptergosidol B (2)

Colorless oil;$[\alpha]_D^{25}$ −150.4 (*c* 0.1 CH_3OH); UV (CH_3OH) λ_{max} (log ε) 200.8 (2.49) nm, 239.0 (2.68); IR (microscope) ν_{max} 3381, 2975, 1645, 1374, 1076, 1036, 970, 923, and 631 cm^{-1}; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$) 200 (+1.48), 214.5 (−1.10); ^{1}H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz), see Table 2; HRESIMS *m/z*439.2314 $[M + Na]^+$ (calcd for $C_{21}H_{36}NaO_8$, 439.2302).

3.6.3. Triptergosidol C (3)

Colorless oil;$[\alpha]_D^{25}$ −183.6 (*c* 0.1 CH_3OH); UV (CH_3OH) λ_{max} (log

ε) 200.8 (2.84) nm, 235.6 (3.12); IR (microscope) ν_{max} 3362, 2973, 1646, 1378, 1078, 1033, 967, 923, and 632 cm^{-1}; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$) 200 (+1.10), 242 (−0.60); ^1H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz), see Table 2; HRESIMS *m/z*439.2316 $[M + Na]^+$ (calcd for $C_{21}H_{36}NaO_8$, 439.2302).

3.6.4. Triptergosidol D (4)

Colorless oil; $[\alpha]_D^{25}$ −189.0 (*c* 0.1. CH_3OH); UV (CH_3OH) λ_{max} (log ε) 200.8 (2.43) nm, 235.2 (2.85); IR (microscope) ν_{max} 3370, 2972, 1644, 1377, 1077, 1035, 967, 922, and 637 cm^{-1}; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$) 200 (+0.80), 240.5 (+1.01); ^1H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz), see Table 2; HRESIMS *m/z*439.2306 $[M + Na]^+$ (calcd for $C_{21}H_{36}NaO_8$, 439.2302).

3.6.5. Triptergerol A

^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Table 1; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$) 317.5 (+ 0.11), 406.5 (+0.25).

3.6.6. Triptergerol B

^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Table 3.

3.6.7. Triptergerol C

^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Table 3.

3.7. Biological activities

Test method of compounds for cytotoxicity against two cell lines, A549 (human lung carcinoma) and Hela, was on basis of previously described [17,18]. Activity of compounds NO production in RAW264.7 macrophage was according to described in the literature [19,20].

4. Conclusion

The present investigation reported the isolation of four compounds (**1**-**4**) and their aglycons (**1**a-**3**a) by Hydrolysis of **1**-**3** with snailase were new nerolidol-type sesquiterpene. There has been no report about the isolation of in *Tripterygiun* genus so far. However, some nerolidol-type sesquiterpenes have been isolated from the genus *euonymus*, Celastraceae family [21], which was further confirmed the close connection between the genus *euonymus* and *tripterygium*. This phytochemical investigation also helped us to enrich our understanding of the chemical constituents of *T. wilfordii*.

Among them, compounds **1**-**4** were evaluated for *in vitro* cytotoxicity against two cell lines, including A549 and Hela, and inhibitory effects against NO production in RAW264.7 macrophages. However, four new nerolidol-type sesquiterpene glucosides showed no cytotoxicity against any of the tumor cell lines, and compounds **1** and **2** showed moderate inhibitory activity against NO production in RAW264.7 macrophages with inhibition ratio value of 65.2 ± 7.3% and 58.2 ± 5.6% at concentration 60 μM, respectively.

Conflict of interest

The authors declare no compete in financial interests.

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Appendix A. Supplementary data

Copies of spectra of compounds **1**-**4** and **1**a-**3**a. Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2018.05.018.

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