New Abietane and Kaurane Type Diterpenoids from the Stems of *Tripterygium regelii*

Dongsheng Fan 1, Shuangyan Zhou 2, Zhiyuan Zheng 1, Guo-Yuan Zhu 1, Xiaojun Yao 1,2, Ming-Rong Yang 1, Zhi-Hong Jiang 1 and Li-Ping Bai 1,*

1 State Key Laboratory of Quality Research in Chinese Medicine, Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Taipa, Macau 999078, China; fandongsheng1985@sina.com (D.F.); zyzheng@must.edu.mo (Z.Z.); gyzhu@must.edu.mo (G.-Y.Z.); xjyao@must.edu.mo (X.Y.); mryang@must.edu.mo (M.-R.Y.); zhjiang@must.edu.mo (Z.-H.J.)

2 State Key Laboratory of Applied Organic Chemistry and Department of Chemistry, Lanzhou University, Lanzhou 730000, China; zhoushy13@lzu.edu.cn

* Correspondence: lpbai@must.edu.mo; Tel.: +853-8897-2403; Fax: +853-2888-0091

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Abstract: Eleven new abietane type (1-11), and one new kaurane (12), diterpenes, together with eleven known compounds (13–23), were isolated and identified from the stems of *Tripterygium regelii*, which has been used as a traditional folk Chinese medicine for the treatment of rheumatoid arthritis in China. The structures of new compounds were characterized by means of the interpretation of high-resolution electrospray ionization mass spectrometry (HRESIMS), extensive nuclear magnetic resonance (NMR) spectroscopic data and comparisons of their experimental CD spectra with calculated electronic circular dichroism (ECD) spectra. Compound 1 is the first abietane type diterpene with an 18→1 lactone ring. Compound 19 was isolated from the plants of the *Tripterygium* genus for the first time, and compounds 14–17 were isolated from *T. regelii* for the first time. Triregelin I (9) showed significant cytotoxicity against A2780 and HepG2 with IC_{50} values of 5.88 and 11.74 µM, respectively. It was found that this compound was inactive against MCF-7 cells. The discovery of these twelve new diterpenes not only provided information on chemical substances of *T. regelii*, but also contributed to the chemical diversity of natural terpenoids.

Keywords: *Tripterygium regelii*; diterpenoids; cytotoxicity

1. Introduction

Diterpenes are naturally-occurring 20-carbon terpenoids that display a wide array of potentially useful biological effects. Abietanes are a large group of diterpenoids, which have been isolated from a variety of terrestrial plants, such as families of Araucariaceae, Cupressaceae, Phyllocladaceae, Pinaceae, Podocarpaceae, Asteraceae, Celastraceae, Hydrocharitaceae, and Lamiaceae, etc. [1,2]. Furthermore, this class of diterpenes have been found from fungal species [2]. So far, it has been reported that some abietane type diterpenes displayed a broad spectrum of promising biological activities including anticancer [3], cytotoxic [4,5], antiviral [6–9], anti-inflammatory [10], and anti-oxidant [9] effects, and so on. For example, tanshinone IIA was regarded as a potent cytotoxic compound for human leukemia cells [11]. Carnosol has been found to possess favorable anticancer and chemo-preventive effects [12]. Triptolide is a promising lead compound to treat inflammatory, immunological and cancerous diseases [13]. Recently, it was reported that miltirone is an inhibitor of P-glycoprotein [14].

As a part of ongoing research work on bioactive constituents from *Tripterygium regelii* [15–17], the methanolic extract of the stems of *T. regelii* was further investigated, leading to the isolation and characterization of twenty three diterpenoids, including eleven new abietane (1–11) and one new
kaurane type (12) diterpenes, as well as eleven known abietane compounds (13–23) (Figure 1). Herein, this paper reports the isolation and structural elucidation of these new diterpenes, as well as cytotoxic evaluation of seventeen diterpenes on three cancer cell lines.

![Chemical structures of compounds 1-23.](image)

**Figure 1.** The chemical structures of compounds 1–23.

### 2. Results and Discussion

Compound 1 was obtained as a yellow amorphous powder with a molecular formula of C_{20}H_{22}O_{5}, which was determined by a protonated molecular ion at m/z 343.1546 [M + H]^{+} (calcd for C_{20}H_{22}O_{5}, 343.1540) in its high-resolution electrospray ionization mass spectrometry (HRESIMS), indicating 10 degrees of unsaturation. IR spectrum of 1 showed a lactone carbonyl band at 1732 cm\(^{-1}\) and benzoquinone bands at 1680 and 1601 cm\(^{-1}\). The UV spectrum of 1 exhibited an absorption maximum at 260 nm, which is characteristic of a p-benzoquinone. The \(^1\)H nuclear magnetic resonance (NMR) spectroscopic data (Table 1) exhibited the characteristic signals for a benzoquinone proton (δ\(_{\text{H}}\) 6.41 (1H, d, J = 1.2 Hz, H-12)), an oxygenated methine (δ\(_{\text{H}}\) 5.86 (1H, d, J = 6.0 Hz, H-1)), an isopropyl moiety including a methine (δ\(_{\text{H}}\) 3.00 (1H, sept d, J = 6.6, 1.2 Hz, H-15)) and two secondary methyls (δ\(_{\text{H}}\) 1.12 and 1.11 (each 3H, d, J = 6.6 Hz, H-16 and H-17)), and two tertiary methyls (δ\(_{\text{H}}\) 1.85 and 1.54 (each 3H, s, H-19 and H-20)). The \(^{13}\)C NMR spectroscopic data (Table 2) displayed resonances for 20 carbons, which were confirmed by distortionless enhancement by polarization transfer (DEPT) and heteronuclear single quantum coherence (HSQC) experiments to be an ester carbonyl carbon (δ\(_{\text{C}}\) 177.3), a trisubstituted p-benzoquinone (δ\(_{\text{C}}\) 187.3 (C-11), 186.8 (C-14), 153.9 (C-13), 146.0 (C-8), 144.6 (C-9) and 131.6 (C-12)), a tetrasubstituted double bond (δ\(_{\text{C}}\) 132.6 (C-5) and 130.2 (C-4)), two aliphatic quaternary carbons (including an oxygenated one), two methines (including an oxygenated...
one), three methylenes, and four methyl groups. These spectroscopic data (Tables 1 and 2) suggested that compound 1 is an abietane type diterpene with a p-benzoquinone C-ring [18,19], structurally similar to the known triptoquinone A (20) [18], an 18(4→3)-abeo-abietane quinone type diterpene, except for the A-ring. The $\Delta^{4,5}$ double bond was inferred from the HMBC correlations from H$_2$-2, H$_2$-6, and H$_3$-19 to C-4 ($\delta_C$ 130.2), from H-7 ($\delta_H$ 2.90), H$_3$-19 and H$_3$-20 to C-5 ($\delta_C$ 132.6). The oxygenated methine ($\delta_H$ 5.86; $\delta_C$ 78.4) was assigned to C-1 based on the HMBC correlations from H-1 proton ($\delta_H$ 5.86) to C-2 ($\delta_C$ 39.1), C-3 ($\delta_C$ 74.3), C-5 ($\delta_C$ 132.6), C-9 ($\delta_C$ 144.6), C-10 ($\delta_C$ 44.7), and C-20 ($\delta_C$ 23.7). The key HMBC correlation from H-1 ($\delta_H$ 5.86) to C-18 ($\delta_C$ 177.3) suggested a lactone formed between C-1 and C-18, accounting for the remaining one degree of unsaturation. Hydroxylation of C-3 was inferred from the HMBC correlations from H-1 ($\delta_H$ 5.86), H$_2$-2 ($\delta_H$ 2.36 and 1.96) and H$_3$-19 ($\delta_H$ 1.85) to C-3 ($\delta_C$ 74.3). It was deduced that the proton at C-1 and the hydroxyl group at C-3 should be a cis relationship due to the lactone between C-1 and C-3. Therefore, the proposed structure of 1 was established as a lactone derivative of triptoquinone A bearing 5S, 10S absolute configuration by X-ray crystallographic analysis [18] (Figure 2).

However, the relative configuration of the substituents at the C-1 and C-3 could not be assigned by nuclear Overhauser effect spectroscopy (NOESY) experiment, owing to the fact that no any key NOE effects were observed (Figure 3). Hence, electron circular dichroism (ECD) calculations were conducted to determine the absolute configuration of compound 1 by time-dependent density functional theory (TDDFT) with the B3LYP/DGDZVP method [20,21]. The calculated ECD of (1R, 3R)-1 matched well with the experimental CD spectrum (Figure 4A) of 1. Therefore, compound 1 was determined as proposed, and given the trivial name of triregelin A.
Table 1. $^1$H NMR (600 MHz) spectroscopic data for compounds 1–5 and 12.

| Position | 1 $^a$ | 2 $^a$ | 3 $^a$ | 4 $^a$ | 5 $^a$ | 12 $^a$ |
|----------|--------|--------|--------|--------|--------|--------|
| 1        | 5.86, d (6.0) | 1.45, m $^c$ | 1.89, ddd (14.4, 10.2, 5.4) | 1.90, m | 1.65, m | 0.79, td (13.2, 3.6) |
|          | -      | 2.77, m $^c$ | 2.80, ddd (14.4, 9.0, 6.0) $^c$ | 2.75, m $^c$ | 2.77, m $^c$ | 1.79, m $^c$ |
| 2        | 1.96, d (10.8) | 2.46, m | 2.46, m $^c$ | 2.45, m $^c$ | 2.52, ddd (15.6, 6.6, 3.6) | 1.43, dt (13.8, 3.6) |
|          | 2.36, m $^c$ | 2.55, m | 2.73, ddd (16.2, 10.2, 6.0) $^c$ | 2.75, m $^c$ | 3.03, ddd (15.6, 7.2, 3.6) $^c$ | 1.55, m $^c$ |
| 3        | -      | -      | -      | -      | -      | 0.95, ddd (13.8, 4.2) |
| 5        | -      | 2.22, m $^c$ | 2.46, d (13.8) $^c$ | 2.47, d (13.8) $^c$ | 1.78, ddd (12.6, 1.8) | 1.00, m $^c$ |
| 6        | 2.16, m | 1.50, m $^c$ | 1.65, td (13.8, 4.2) | 1.41, td (13.8, 3.0) | 1.59, m | 1.36, qd (12.6, 3.6) |
|          | 2.76, ddd (13.2, 6.0) | 2.24, m $^c$ | 1.96, br d (13.8) | 2.02, dt (13.8, 1.8) | 1.96, br d, d (13.2, 7.2) | 1.72, m $^c$ |
| 7        | 2.37, m $^c$ | 2.39, ddd (18.6, 11.4, 7.2) | 4.81, br s | 4.39, ddd (3.0, 1.8) | 2.34, ddd (18.6, 12.0, 7.2) | 1.62, dd (13.8, 4.8) |
|          | 2.90, ddd (19.8, 6.0) | 2.80, m | - | - | 2.81, m $^c$ | 1.72, m $^c$ |
| 9        | -      | -      | -      | -      | -      | -      |
| 11       | -      | -      | -      | -      | -      | 2.24, d (17.0) |
| 12       | 6.41, d (1.2) | 6.47, s | 6.44, s | 6.42, d (1.2) | 6.37, d (1.2) | -      |
| 13       | -      | -      | -      | -      | -      | 3.21, d (4.8) |
| 14       | -      | -      | -      | -      | -      | 2.40, d (12.6) |
| 15       | 3.00, sept d (6.6, 1.2) | 3.12, m | 3.02, sept (7.0) | 3.04, sept d (7.2, 1.2) | 3.00, d (7.2) $^c$ | 2.36, s |
| 16       | 1.12, d (6.6) | 1.17, d (7.2) | 1.14, d (7.0) | 1.12, d (7.2) | 1.10, d (7.2) | -      |
| 17       | -      | 3.67, d (7.2) | 1.13, d (7.0) | 1.13, d (7.2) | 1.11, d (7.2) | 4.87, s |
| 18       | -      | -      | 1.37, s | 1.36, s | 1.22, s | 0.98, s |
| 19       | 1.85, s | 2.11, s | 3.47, t (10.8) | 3.44, d (12.0) | 4.56, d (12.0) | 3.68, dd (10.1, 4.0) |
|          | -      | -      | 4.02, ddd (10.8, 2.4) | 4.05, d (12.0) | 4.08, d (12.0) | 3.44, dd (10.1, 4.0) |
| 20       | 1.54, s | 1.18, s | 1.24, s | 1.22, s | 1.44, s | 0.85, s |
| OH-7     | -      | -      | 2.77, s $^c$ | -      | -      | -      |
| OMe-7    | -      | -      | 3.15, ddd (10.8, 2.4) | 3.26, br s | -      | 1.09, br s |
| OAc-19   | -      | -      | 3.50, s | -      | -      | -      |

$^a$ Measured in CDCl$_3$; $^c$ Overlapping signal was assigned from $^1$H–$^1$H COSY, HSQC and HMBC experiments. The signals of br, s, d, t, q, sept and m represent broad, singlet, doublet, triplet, quartet, septet and multiplet splitting patterns of protons, respectively.
Table 2. $^{13}$C NMR (150 MHz) spectroscopic data for compounds 1–12.

| Position | $\delta_{C}$, Type |
|----------|-------------------|
|          | 1 $^a$ | 2 $^a$ | 3 $^a$ | 4 $^a$ | 5 $^a$ | 6 $^a$ | 7 $^a$ | 8 $^a$ | 9 $^a$ | 10 $^a$ | 11 $^b$ | 12 $^a$ |
| 1 | 78.4, CH | 31.8, CH$_2$ | 34.1, CH$_2$ | 34.1, CH$_2$ | 34.7, CH$_2$ | 38.2, CH$_2$ | 35.1, CH$_2$ | 35.1, CH$_2$ | 34.5, CH$_2$ | 36.2, CH$_2$ | 32.8, CH$_2$ | 39.7, CH$_2$ |
| 2 | 39.1, CH$_2$ | 24.6, CH$_2$ | 34.2, CH$_2$ | 34.2, CH$_2$ | 34.9, CH$_2$ | 27.4, CH$_2$ | 35.3, CH$_2$ | 34.8, CH$_2$ | 28.3, CH$_2$ | 28.1, CH$_2$ | 27.0, CH$_2$ | 17.8, CH$_2$ |
| 3 | 74.3, C | 147.9, C | 220.4, C | 220.8, C | 212.4, C | 46.1, CH | 214.8, C | 219.0, C | 79.7, CH | 79.9, CH | 74.8, CH | 35.5, CH$_2$ |
| 4 | 130.2, C | 124.5, C | 49.9, C | 49.7, C | 51.3, C | 150.8, C | 53.0, C | 50.3, C | 42.8, C | 42.3, C | 38.3, C | 38.6, C |
| 5 | 332.6, C | 47.3, CH | 45.2, CH | 44.9, CH | 52.9, CH | 47.9, CH | 51.5, CH | 49.4, CH | 49.6, CH | 48.8, CH | 44.0, CH | 56.2, CH |
| 6 | 22.2, CH$_2$ | 18.7, CH$_2$ | 26.1, CH$_2$ | 22.5, CH$_2$ | 18.5, CH$_2$ | 20.8, CH$_2$ | 124.0, CH | 35.6, CH$_2$ | 35.7, CH$_2$ | 18.9, CH$_2$ | 20.2, CH$_2$ |
| 7 | 270.0, CH$_2$ | 252.5, CH$_2$ | 61.9, CH | 69.8, CH | 26.0, CH$_2$ | 23.7, CH$_2$ | 122.1, CH | 204.3, C | 205.6, C | 205.2, C | 25.1, CH$_2$ | 39.4, CH$_2$ |
| 8 | 146.0, C | 149.0, C | 140.9, C | 139.3, C | 142.8, C | 120.7, C | 113.6, C | 114.9, C | 115.1, C | 114.2, C | 119.7, C | 44.1, C |
| 9 | 144.6, C | 149.0, C | 148.7, C | 148.6, C | 148.0, C | 145.8, C | 145.2, C | 133.1, C | 134.7, C | 153.1, C | 150.0, C | 57.6, CH |
| 10 | 44.7, C | 36.6, C | 37.5, C | 37.3, C | 37.6, C | 39.7, C | 37.7, C | 38.3, C | 39.5, C | 37.5, C | 37.9, C | 39.2, C |
| 11 | 187.3, C | 187.4, C | 187.9, C | 187.5, C | 117.6, CH | 98.4, CH | 144.5, C | 144.2, C | 113.6, CH | 108.8, CH | 35.9, CH$_2$ |
| 12 | 131.6, CH | 134.0, CH | 132.4, CH | 131.8, CH | 132.0, CH | 123.3, CH | 158.3, CH | 123.8, CH | 123.9, CH | 133.6, CH | 156.5, CH | 211.5, CH |
| 13 | 153.9, C | 149.0, C | 153.6, C | 154.0, C | 153.3, C | 130.3, C | 119.6, C | 136.6, C | 136.3, C | 134.9, C | 125.1, C | 60.7, CH |
| 14 | 186.8, C | 188.1, C | 188.7, C | 186.4, C | 187.4, C | 150.3, C | 150.3, C | 155.4, C | 155.7, C | 160.7, C | 156.9, C | 39.4, CH$_2$ |
| 15 | 26.6, CH | 34.5, CH | 26.4, CH | 26.5, CH | 26.4, CH | 26.9, CH | 24.3, CH | 26.0, CH | 26.0, CH | 26.1, CH | 26.1, CH | 48.2, CH |
| 16 | 21.3, CH$_3$ | 15.4, CH$_3$ | 21.3, CH$_3$ | 21.3, CH$_3$ | 21.3, CH$_3$ | 22.6, CH$_3$ | 20.9, CH$_3$ | 22.1, CH$_3$ | 22.1, CH$_3$ | 22.1, CH$_3$ | 21.6, CH$_3$ | 148.8, C |
| 17 | 21.4, CH$_3$ | 66.6, CH$_2$ | 21.3, CH$_3$ | 21.4, CH$_3$ | 21.3, CH$_3$ | 22.8, CH$_3$ | 20.9, CH$_3$ | 22.2, CH$_3$ | 22.2, CH$_3$ | 22.3, CH$_3$ | 21.7, CH$_3$ | 107.8, CH$_2$ |
| 18 | 177.3, C | 173.7, C | 22.3, CH$_3$ | 22.3, CH$_3$ | 21.8, CH$_3$ | 64.7, CH$_2$ | 19.7, CH$_3$ | 22.6, CH$_3$ | 22.4, CH$_3$ | 22.0, CH$_3$ | 29.1, CH$_3$ | 26.9, CH$_3$ |
| 19 | 118.3, CH$_3$ | 185.5, CH$_3$ | 65.7, CH$_2$ | 65.8, CH$_2$ | 65.7, CH$_2$ | 104.5, CH$_2$ | 65.9, CH$_2$ | 65.5, CH$_2$ | 63.7, CH$_2$ | 63.7, CH$_2$ | 22.4, CH$_3$ | 65.4, CH$_3$ |
| 20 | 23.7, CH$_3$ | 19.2, CH$_3$ | 19.7, CH$_3$ | 20.0, CH$_3$ | 20.2, CH$_3$ | 22.5, CH$_3$ | 20.3, CH$_3$ | 18.3, CH$_3$ | 18.3, CH$_3$ | 24.2, CH$_3$ | 25.2, CH$_3$ | 16.4, CH$_3$ |

$^a$ Measured in CDCl$_3$, $^b$ Measured in pyridine-$d_5$. 
Compound 2 was assigned as a molecular formula of $C_{20}H_{24}O_5$ based on a deprotonated molecular ion at $m/z$ 343.1560 [M − H]$^-$ (calcd for $C_{20}H_{23}O_5$, 343.1551) in its HRESIMS. IR spectrum of 2 exhibited a conjugated carboxylic acid band at 1688 cm$^{-1}$ and benzoquinone band at 1649 cm$^{-1}$. The $^1$H and $^{13}$C NMR spectroscopic data (Tables 1 and 2) of 2 were closely analogous to those of triptoquinone A (20) [18], except for the absence of a secondary methyl group and the presence of a hydroxymethyl group ($\delta_H$ 3.67 (2H, d, $J = 7.2$ Hz, H$_2$-17); $\delta_C$ 66.6). The hydroxymethyl group was allocated to be at C-15, as deduced by $^1$H-$^1$H COSY correlation of H-15/H$_2$-17 and HMBC correlations from the hydroxymethyl protons to C-13 ($\delta_C$ 149.0) and C-15 ($\delta_C$ 34.5). Therefore, compound 2 was characterized and given a trivial name of triregelin B.

Compound 3 showed a molecular formula of $C_{20}H_{26}O_5$, as established from an [M + H]$^+$ ion at $m/z$ 347.1866 (calcd $C_{20}H_{27}O_5$, 347.1853) in the HRESIMS. Analysis of the NMR spectroscopic data...
(Tables 1 and 2) indicated that 3 was structurally related to triptoquinone B (21) [18] except for the absence of the C-7 methylene in triptoquinone B and the presence of an additional hydroxyl proton (δ_H 2.77) and an oxygenated methine (δ_C 4.81, δ_C 61.9) in 3. These data suggested hydroxylation of C-7 in 3, which was supported by HMBC correlation from the hydroxyl proton (δ_H 2.77) to C-7 (δ_C 61.9). The α-orientation of the hydroxyl group at C-7 was deduced from the NOESY correlation of H-7/H3-20. Thus, compound 3 was identified and named triregelin C.

Compound 4 gave a molecular formula of C_{21}H_{25}O_{5}, as deduced from an [M + H]^+ ion at m/z 361.2008 (calcd for C_{21}H_{25}O_{5}, 361.2010), 14.0142 atomic mass units (amu) more than that of 3 in the HRESIMS. The 1^H and 13^C NMR spectroscopic data (Tables 1 and 2) of 4 were closely similar to those of 3, except for the appearance of a methoxyl group. The methoxyl group was assigned at C-7, as evidenced from the observed HMBC correlation from the methoxyl protons (δ_H 3.50) to C-7 (δ_C 69.8). Thus, compound 4 was characterized and named triregelin D.

Compound 5 showed a molecular formula of C_{22}H_{25}O_{5} on the basis of a protonated molecular ion at m/z 373.1998 [M + H]^+ (calcd for C_{22}H_{25}O_{5}, 373.2010) in its HRESIMS, 42.0106 amu more than that of triptoquinone B (21) [18]. The 1D NMR spectroscopic data (Tables 1 and 2) of 5 were analogous to those of triptoquinone B (21) [18], except for the presence of an acetyl group (δ_H 2.03, δ_C 170.8, 20.9). The acetyl group was allocated to C-19, as evidenced from the HMBC correlations from H2-19 (δ_H 4.56, 4.08) to the carbonyl carbon (δ_C 170.8) of the acetyl group. Therefore, compound 5 was determined and named triregelin E.

Compound 6 had a molecular formula of C_{20}H_{24}O_{5} deduced from a protonated molecular ion at m/z 301.2162 [M + H]^+ (calcd for C_{20}H_{24}O_{5}, 301.2162) in the HRESIMS. IR spectrum of 6 displayed a double bond band at 1626 cm⁻¹, and aromatic ring bands at 1580 and 1424 cm⁻¹. The 1^H NMR data (Table 3) showed the characteristic signals for two coupled aromatic protons (δ_H 7.04 and 6.09 (1H each, d, J = 7.8 Hz)), two singlet vinylic protons (δ_H 4.86 and 4.77), an oxygenated methylene (δ_H 3.95 and 3.70), an isopropyl moiety (δ_H 3.15, 1.26 and 1.25), and a tertiary methyl group (δ_H 0.99). The 13^C NMR data (Table 2) displayed resonances for 20 carbons, which were ascribed to a tetrasubstituted benzene ring, an exocyclic double bond, an aliphatic quaternary carbon, three methines, five methylenes (including an oxygenated one) and three methyl groups. The 1^H and 13^C NMR spectroscopic data of 6 were similar to those of triptobenzene P [22], an 18 (4 → 3)-abeo-abietane diterpene previously isolated from T. wilfordii, except for the following two differences. One difference is the replacement of the methoxyl group at C-12 in triptobenzene P by a hydrogen in 6, which was supported by 1H–1H COSY correlation of H-11/H-12, and HMBC correlations from H-12 (δ_H 7.04) to C-9 (δ_C 145.8) and C-15 (δ_C 26.9). The other difference is the downfield shift of C-14 (δ_C 150.3) in 6 relative to that (δ_C 123.8) in triptobenzene P, indicating hydroxylation of C-14 in 6. Thus, the planar structure of 6 was established as 12-demethoxy-14-hydroxy-triptobenzene P, which was confirmed by the 1H–1H COSY and HMBC data (Figure 2). The NOE correlations of H-5α/H-21-18 and H2-20β/H-3 indicated α-orientation of CH2-18 (Figure 3). Thus, compound 6 was defined and named triregelin F.

Compound 7 gave a molecular formula of C_{21}H_{25}O_{5}, as established from an [M + H]^+ ion at m/z 345.2055 (calcd for C_{21}H_{25}O_{5}, 345.2060) in the HRESIMS. IR spectrum of 7 exhibited a carbonyl band at 1703 cm⁻¹, and aromatic ring bands at 1604, 1566 and 1455 cm⁻¹. The 1^H and 13C NMR spectroscopic data (Tables 2 and 3) of 7 were very similar to those of triptobenzene A (13) [23], except for the absence of two methylene groups and an aromatic proton, and the presence of a double bond (δ_H 6.82 (1H, dd, J = 10.2, 3.0 Hz, H-7); 122.1 and 5.86 (1H, dd, J = 10.2, 3.0 Hz, H-6); δ_C 124.0), and a methoxyl group (δ_H 3.80 (3H, s, OCH3-12); δ_C 55.7). The double bond was assigned at between C-6 and C-7, which was supported by HMBC correlations from H-6 (δ_H 5.86) to C-4 (δ_C 53.0) and C-10 (δ_C 37.7), and from H-7 (δ_H 6.82) to C-9 (δ_C 145.2) and C-14 (δ_C 150.3). The methoxyl group was located at C-12, as deduced from the HMBC correlation from the methoxyl protons (δ_H 3.80) to C-12 (δ_C 158.3). The key NOE correlations of H-5α/H-21-18 and H2-20β/H-19 were observed in the NOESY spectrum. Accordingly, compound 7 was elucidated as illustrated in Figure 1, and named triregelin G.
Table 3. $^1$H NMR (600 MHz) spectroscopic data for compounds 6–11.

| Position | 6 $^a$ | 7 $^a$ | 8 $^a$ | 9 $^a$ | 10 $^a$ | 11 $^b$ |
|----------|-------|-------|-------|-------|-------|-------|
| 1        | 1.63, td (13.2, 4.2) | 2.14, td (13.2, 5.4) | 2.07, ddd (16.2, 9.6, 4.8) | 1.50, td (13.8, 3.6) | 1.70, td (13.8, 4.2) | 1.97, td (12.6, 3.6) |
| 2        | 2.32, dt (13.2, 4.2) | 2.48, ddd (12.6, 6.0, 3.0) | 3.31, m $^c$ | 3.34, dt (13.8, 3.6) | 2.36, dt (13.8, 3.0) | 2.38, td (13.2, 3.6) |
| 3        | 1.43, qd (13.0, 4.2) | 192, m $^c$ | 2.61, ddd (15.6, 5.4, 3.0) | 2.54, ddd (15.6, 8.4, 7.2) | 1.89, m $^c$ | 1.98, m $^c$ | 1.87, m $^c$ |
| 4        | 2.20, m $^c$ | 2.69, d (15.0) $^c$ | 2.64, d (15.0) $^c$ | 1.91, dd (14.4, 2.4) $^c$ | 1.94, dd (14.4, 3.6) | 3.55, dd (11.4, 3.6) | 3.68, q (3.6) |
| 5        | 2.17, d (12.6) $^c$ | - | 5.86, dd (10.2, 3.0) | 2.63, d (16.2) | 2.64, dd (16.8, 14.4) | 2.67, dd (18.0, 14.4) | 1.88, m $^c$ |
| 6        | 1.81, qd (12.6, 6.0) | 195, m $^c$ | 6.82, dd (10.2, 3.0) | 2.72, m $^c$ | 2.74, dd (16.8, 14.4) | 2.80, dd (18.0, 14.4) | 1.72, m $^c$ |
| 7        | 2.61, ddd (16.4, 12.6, 7.2) | 2.87, dd (16.4, 6.0) | - | - | - | - | 2.81, ddd (16.2, 11.4, 7.8) |
| 8        | 2.74, dd (16.2, 11.4, 7.8) | - | - | - | - | - | 3.13, dd (16.2, 6.6) |
| 9        | 5.86, dd (10.2, 3.0) | 2.63, d (16.2) | 2.64, dd (16.8, 14.4) | 2.67, dd (18.0, 14.4) | 1.88, m $^c$ | 1.72, m $^c$ |
| 10       | 5.86, dd (10.2, 3.0) | 2.63, d (16.2) | 2.64, dd (16.8, 14.4) | 2.67, dd (18.0, 14.4) | 1.88, m $^c$ | 1.72, m $^c$ |
| 11       | 2.20, m $^c$ | 2.69, d (15.0) $^c$ | 2.64, d (15.0) $^c$ | 1.91, dd (14.4, 2.4) $^c$ | 1.94, dd (14.4, 3.6) | 3.55, dd (11.4, 3.6) | 3.68, q (3.6) |
| 12       | 2.17, d (12.6) $^c$ | - | 5.86, dd (10.2, 3.0) | 2.63, d (16.2) | 2.64, dd (16.8, 14.4) | 2.67, dd (18.0, 14.4) | 1.88, m $^c$ |
| 13       | 1.81, qd (12.6, 6.0) | 195, m $^c$ | 6.82, dd (10.2, 3.0) | 2.72, m $^c$ | 2.74, dd (16.8, 14.4) | 2.80, dd (18.0, 14.4) | 1.72, m $^c$ |
| 14       | 2.61, ddd (16.4, 12.6, 7.2) | 2.87, dd (16.4, 6.0) | - | - | - | - | 2.81, ddd (16.2, 11.4, 7.8) |
| 15       | 2.74, dd (16.2, 11.4, 7.8) | - | - | - | - | - | 3.13, dd (16.2, 6.6) |
| 16       | 5.86, dd (10.2, 3.0) | 2.63, d (16.2) | 2.64, dd (16.8, 14.4) | 2.67, dd (18.0, 14.4) | 1.88, m $^c$ | 1.72, m $^c$ |
| 17       | 5.86, dd (10.2, 3.0) | 2.63, d (16.2) | 2.64, dd (16.8, 14.4) | 2.67, dd (18.0, 14.4) | 1.88, m $^c$ | 1.72, m $^c$ |
| 18       | 3.70, dd (10.8, 6.0) | 1.27, s | 1.35, s | 1.30, s | 1.29, s | 1.27, s |
| 19       | 3.95, dd (10.8, 6.0) | - | - | - | - | - |
| 20       | 3.70, dd (10.8, 6.0) | 1.27, s | 1.35, s | 1.30, s | 1.29, s | 1.27, s |
| OH-3     | 4.77, s | 3.84, d (12.0, 4.8) | 3.54, d (11.4) | 3.42, dd (11.4, 9.0) | 3.50, dd (11.4, 7.8) | 0.96, s |
| OH-11    | 4.86, s | 4.14, d (12.0) | 4.06, d (11.4) | 4.35, d (11.4) | 4.36, d (11.4) | - |
| OH-12    | - | - | - | - | - | - |
| OH-14    | - | - | - | - | - | - |
| OH-19    | - | - | - | - | - | - |
| OMe-12   | - | - | - | - | - | - |
| OMe-14   | - | - | - | - | - | - |

$^a$ Measured in CDCl$_3$; $^b$ Measured in pyridine-$_d_5$; $^c$ Overlapping signal was assigned from $^1$H–$^1$H COSY, HSQC, and HMBC experiments. The signals of br, s, d, t, q, sept and m represent broad, singlet, doublet, triplet, quartet, septet and multiplet splitting patterns of protons, respectively.
Compound 8 had a molecular formula of C_{20}H_{26}O_{5}, according to an [M + H]^+ ion at m/z 347.1840 [M + H]^+ (calcd for C_{20}H_{26}O_{5}, 347.1853). The 1^H and 13C NMR spectroscopic data (Tables 2 and 3) of 8 were closely related to those of triptobenzene A (13) [23]. However, one of the key differences was the replacement of the methylene at C-7 in triptobenzene A by a keto carbonyl carbon (δC 204.3) in 8, as evidenced from HMBC correlation from H-5 (δH 2.64) to C-7. The other difference was the absence of a doublet aromatic proton and the presence of an additional hydroxyl proton (δH 4.62) together with the downfield shift of C-11 (δC 144.5) in 8 compared to that in triptobenzene A, which suggested hydroxylation of C-11 in 8. Therefore, compound 8 was assigned and named triregelin H.

Compound 9 had a molecular formula of C_{20}H_{20}O_{3} based on a protonated molecular ion at m/z 349.2005 [M + H]^+ (calcd for C_{20}H_{20}O_{3}, 349.2010), with 2.0161 amu more than that of 8 in the HRESIMS. The 13C NMR spectroscopic data (Table 2) of 9 were closely comparable to those of 8, except for the absence of the C-3 keto carbonyl in 8, and the presence of an oxygenated methine (δH 3.56 (1H, dd, J = 11.4, 3.6 Hz, H-3); δC 79.7) in 9. These suggested that the C-3 keto carbonyl group in 8 was reduced to be a hydroxyl group in 9. The hydroxyl group at C-3 was α-oriented, as deduced from the NOESY correlations of H-3/H-5α and H-3/H-3β-18. Therefore, compound 9 was identified and named triregelin I.

Compound 10 displayed a molecular formula of C_{20}H_{28}O_{4} established by a protonated molecular ion at m/z 333.2053 [M + H]^+ (calcd for C_{20}H_{28}O_{4}, 333.2060), revealing 15.9948 amu less than that in 9 in the HRESIMS. The 1H and 13C NMR spectroscopic data (Tables 2 and 3) of 10 were very similar to those of 9 except for the presence of an extra doublet aromatic proton (δH 6.74, H-11) and the upfield shift of C-11 (δC 113.6) relative to that (δC 144.2) in 9. These data revealed the dehydroxylation of C-11 in 10, which was further supported by 1H–1H COSY correlation of H-11/H-12, and HMBC correlations from H-11 to C-8 (δC 114.2), C-10 (δC 37.5), and C-13 (δC 134.9). Hence, compound 10 was elucidated and named triregelin J.

Compound 11 showed a molecular formula of C_{21}H_{32}O_{3}, as deduced from a protonated molecular ion at m/z 333.2426 [M + H]^+ (calcd for C_{21}H_{32}O_{3}, 333.2424) in the HRESIMS. Comparison of the NMR spectroscopic data (Tables 2 and 3) of 11 with neotriptonoterpene (14) [24] showed that both compounds were structurally comparable, except for the absence of the C-3 keto carbonyl in neotriptonoterpene (14) and the presence of an extra oxygenated methine (δH 3.68; δC 74.8) in 11. These suggested that the C-3 keto carbonyl group in neotriptonoterpene (14) was reduced to be a hydroxyl group in 11. The C-3 hydroxyl group was α-oriented, as inferred from the coupling constant (J_{2,3} = 3.6 Hz) and the NOESY correlation between H-3 and H-3β-19. Accordingly, the compound 11 was characterized and named triregelin K.

Compound 12, white amorphous power, had a molecular formula of C_{20}H_{30}O_{2}, as deduced from an [M + H]^+ ion at m/z 303.2322 (calcd for C_{20}H_{30}O_{2}, 303.2319) in the HRESIMS. IR spectrum of 12 exhibited a strong carbonyl band at 1710 cm⁻¹. The 1H NMR spectrum (Table 1) exhibited the characteristic signals for a vinlylic group (δH 4.99 and 4.87), an oxygenated methylene (δH 3.68 and 3.44), a hydroxyl group (δH 1.09), and two tertiary methyls (δH 0.98 and 0.85). The 13C NMR and DEPT spectra (Table 2) showed 20 carbon signals including a carbonyl group, an exocyclic double bond, three quaternary carbons, three methines, nine methylenes (including an oxygenated one) and two methyl groups. All the above NMR data indicated that 12 was a kaurane type diterpenoid, and structurally similar to (−)-ent-kaur-16-en-19-ol [25–27]. The distinct difference was that the C-12 methylene in (−)-ent-kaur-16-en-19-ol was oxidized to be a keto carbonyl group in 12, as deduced from the downfield shift of C-12 (δC 211.5), and the HMBC correlations from H-9 (δC 1.58) and H-14 (δH 2.40, 1.51) to C-12. Finally, the planar structure of 12 was confirmed on the basis of the 1H–1H COSY and HMBC experiments (Figure 2). In the NOE spectrum, the correlations of H_{2}-20/H_{2}-19 and H_{2}-20/H_{2}-14 indicated that these protons were in the same face. In the same way, the other key NOE cross peaks of H-5/H-9 and H-9/H-2-15 were also observed (Figure 3), suggesting H-5, H-9, and H-2-15 were in the other face. However, 12 displayed a positive specific rotation ([α]_{D}^{20} +50.86 (c 0.50, MeOH)) in contrast to the negative one reported for (−)-ent-kaur-16-en-19-ol [27]. ECD curves for
the two possible stereo-structures (4R, 5S, 8S, 9R, 10S, 13R-12 and 4S, 5R, 8R, 9S, 10R, 13S-12) were, therefore, calculated to determine the absolute configuration of 12. As illustrated in Figure 4B, the calculated profile of 4R, 5S, 8S, 9R, 10S, and 13R-12 were in good agreement with the experimental CD spectrum of 12. Therefore, compound 12 was identified and named triregelin L.

The compounds 3, 6, 7, and 9 were also selected to calculate their ECD data in order to further confirm their absolute configurations. As the results, their experimental CD spectra showed similar CD pattern to the calculated ones of (4S, 5R, 7R, 10S)-3, (3R, 5S, 10S)-6, (4S, 5R, 10S)-7, and (3S, 4S, 5R, 10S)-9, respectively (Figure S1). The HRMS, UV, IR, NMR and CD spectra (Figures S2–S121) of twelve new compounds were shown in supplementary materials.

In addition, eleven known abietanes were also isolated from the stems of T. regelii, including triptobenzene A (13) [23], neotripteronterpe (14) [24], triptobenzene M (15) [28], wilforol F (16) [29], triptobenzene J (17) [30], triptobenzene B (18) [23], abiesta-8, 11, 13-triene-14, 19-diol (19) [31], triptoquinone A (20), triptoquinone B (21), triptoquinone D (22) and triptoquinone F (23) [18]. These compounds were identified by comparison of their spectroscopic (1D NMR and specific rotation) and HRMS data with those reported in the literature. Compound 1 is the first abietane type diterpene with an 18→1 lactone ring. The discovery of the above twelve new diterpenes contributed to the chemical diversity of natural terpenoids.

Cytotoxic effects of seventeen diterpenes (2, 7–11, 13–23) were evaluated against three cancer cell lines of A2780, HepG2 and MCF-7. As the results show (Table 4), compound 9 displayed cytotoxicity against A2780, HepG2, and MCF-7 cells with IC₅₀ values of 5.88, 11.74, and 46.40 µM, respectively. Compound 11 showed solely cytotoxic effect on MCF-7 cell with an IC₅₀ value of 26.70 µM. Compound 14 exhibited weak cytotoxic activity on A2780, HepG2, and MCF-7 cells with IC₅₀ values of 65.80, 35.45, and 64.80 µM, respectively.

Table 4. Cytotoxic effects of diterpenes on three cancer cell lines of A2780, HepG2, and MCF-7.

| Compounds * | IC₅₀ (µM) against A2780 | IC₅₀ (µM) against HepG2 | IC₅₀ (µM) against MCF-7 |
|-------------|-------------------------|-------------------------|-------------------------|
| 9           | 5.88 ± 2.22             | 11.74 ± 1.92            | 46.40 ± 3.54            |
| 11          | >100                    | >100                    | 26.70 ± 5.57            |
| 14          | 65.80 ± 21.53           | 35.45 ± 8.23            | 64.80 ± 24.90           |
| taxol       | 0.006 ± 0.001           | 0.003 ± 0.0002          | 0.005 ± 0.001           |

* Seventeen compounds (2, 7–11, 13–23) were evaluated for cytotoxic effects against three cancer cell lines; IC₅₀ values for other tested compounds were larger than 100 µM on three cancer cells.

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were obtained using a Rudolph Research Analytical Autopol I automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). IR spectra were measured on an Agilent Cary 600 series FT-IR spectrometer (KBr) (Agilent, Santa Clara, CA, USA). Ultraviolet (UV) spectra were recorded on a Beckman Coulter DU® 800 spectrophotometer (Beckman Coulter, Fullerton, CA, USA). HRMS spectra were carried out on an Agilent 6230 electrospray ionization (ESI) time-of-flight (TOF) mass spectrometer (Agilent, Santa Clara, CA, USA). Nuclear magnetic resonance (NMR) spectra were measured on a Bruker Ascend 600 NMR spectrometer at 600 MHz for ¹H NMR and 150 MHz for ¹³C NMR (Bruker, Zurich, Switzerland). Chemical shifts were expressed in δ (ppm) with tetramethylsilane (TMS) as an internal reference, and coupling constants (J) were reported in hertz (Hz). Circular dichroism spectra were measured on a Jasco J1500 CD spectrometer (Jasco Corporation, Tokyo, Japan). Medium pressure liquid chromatography (MPLC) was conducted on a Sepacore Flash Chromatography System (Buchi, Flawil, Switzerland) by employing a flash column (460 mm × 36 mm, i.d., Buchi) packed with Bondapak Waters ODS (40–63 µm, Waters, Milford, MA, USA). Preparative high performance liquid chromatography (HPLC) was carried out on a Waters
Xbridge Prep C₈ column (10 mm × 250 mm, 5 µm) by utilizing a Waters liquid chromatography system equipped with 1525 Binary HPLC Pump and 2489 UV/Visible detector (Waters, Milford, MA, USA). Semi-preparative HPLC was done on a Waters Xbridge Prep C₁₈ column (10 mm × 250 mm, 5 µm) by using an Agilent 1100 liquid chromatography system coupled with a quaternary pump and a diode array detector (DAD) (Agilent, Santa Clara, CA, USA). Column chromatography was conducted on silica gel (40–60 µm, Grace, Columbia, MD, USA) and Bondapak Waters ODS (40–63 µm, Waters). Thin layer chromatographies (TLCs) were performed on pre-coated silica gel 60 F₂₅⁴ plates (200 µm thick, Merck KGaA, Darmstadt, Germany), which were used to monitor fractions. Spots on the TLC were visualized by UV light (254 nm) or heating after spraying with 5% H₂SO₄ in ethanol.

3.2. Plant Material

The stems of *T. regelii* used in this study were collected from Changbai Mountain in Jilin province, China, in October 2012. The plant was authenticated by Liang Xu, Liaoning University of Traditional Chinese Medicine (Dalian, China). A voucher specimen (No. MUST-TR201210) has been deposited at State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology.

3.3. Extraction and Isolation

The air-dried stems of *T. regelii* (8.0 kg) were powdered, and extracted three times with methanol (64 L) under ultrasonic-assisted extraction at room temperature for 1 h. The methanol extract was evaporated under reduced pressure to yield a dark brown residue, which was then suspended in H₂O, and successively partitioned with *n*-hexane, ethyl acetate (EtOAc) and *n*-butanol. Then, the EtOAc-soluble extract (150.0 g) was fractionated over a silica gel column using a gradient system of petroleum ether (PE)-acetone (PE-acetone: 100:0–35:65, v/v) to provide thirteen fractions (Fr.1–Fr.13). Fraction 5 was chromatographed over an ODS column using a gradient system of MeOH–H₂O (50:50–100:0, v/v) to yield eight fractions (Fr.5-1–Fr.5-8). Fractions 5-2 (110.0 mg) and 5-4 (130.0 mg) were further separated on silica gel columns eluted with PE–EtOAc (90:10-30:70, v/v) to afford compounds 22 (15.0 mg) and 23 (30.0 mg), respectively. Similarly, fraction 7 (5.0 g) was subjected to an ODS column with a gradient system of MeOH–H₂O (40:60–90:10, v/v) to give nine fractions (Fr.7-1–Fr.7-9). Compounds 20 (50.0 mg) and 21 (30.0 mg) were obtained by silica gel columns separation using PE–EtOAc (90:10–30:70, v/v) as eluting solvents from fractions 7-2 (150.0 mg) and 7-4 (100.0 mg), respectively. Fraction 8 (5.4 g) was subjected to an ODS column using a gradient system of MeOH–H₂O (35:65–80:20, v/v) to afford ten fractions (Fr.8-1–Fr.8-10). The fraction 8-3 (2.5 g) was fractionated over a silica gel column, with a gradient elution by PE–EtOAc (90:10–63:35, v/v), to produce seven fractions (Fr.8-3-1–Fr.8-3-7). Fraction 8-3-4 (40.5 mg) was purified by semi-preparative HPLC using CH₃CN–H₂O (52:48, v/v) as mobile phase to afford compounds 5 (0.6 mg), 12 (0.7 mg) and 18 (1.0 mg). Fraction 8-3-5 (200.6 mg) was subjected to semi-preparative HPLC using CH₃CN-H₂O (58:42, v/v) as solvent system to give compounds 6 (1.9 mg) and 11 (2.0 mg), as well as subfraction 8-3-5-2. Compounds 14 (1.6 mg) and 19 (1.0 mg) were obtained by preparative HPLC with an isocratic elution of CH₃CN-H₂O (55:45, v/v) from the subfraction 8-3-5-2 (65.7 mg). Fraction 11 (5.5 g) was chromatographed over an ODS column, eluted with MeOH–H₂O (30:70–100:0, v/v), to afford sixteen fractions (Fr.11-1–Fr.11-16). Compound 3 (5.0 mg) was isolated by preparative HPLC eluting with a MeOH–H₂O (35:65, v/v) solvent system from fraction 11-4 (26.9 mg). Fraction 11-6 (49.5 mg) was separated by semi-preparative HPLC using CH₃CN–H₂O (35:65, v/v) as mobile phase to yield compound 2 (5.0 mg). Fraction 11-7 (261.1 mg) was subjected to a silica gel column with a gradient elution of PE–EtOAc (20:80–10:90, v/v) and purified by semi-preparative HPLC using CH₃CN–H₂O (40:60, v/v) as eluting solvent to afford compounds 8 (1.2 mg), 4 (2.0 mg), 13 (1.6 mg) and fraction 11-7-3. Compounds 7 (0.6 mg) and 16 (3.1 mg) were purified by semi-preparative HPLC with a CH₃CN–H₂O (42:58, v/v) solvent system from fraction 11-7-3 (56.9 mg). Fraction 11-8 (284.3 mg) was isolated by preparative HPLC.
using MeOH–H₂O (46:54, v/v) as mobile phase to yield compound 1 (0.59 mg) and five fractions (Fr.11-8-1–Fr.11-8-5). Fraction 11-8-2 (42.5 mg) was subjected to semi-preparative HPLC with a CH₂CN–H₂O (47:53, v/v) solvent system to give compound 15 (2.0 mg). Fraction 11-8-3 (30.3 mg) was isolated by semi-preparative HPLC using CH₂CN–H₂O (45:55, v/v) as eluting solvent to yield compounds 10 (1.6 mg) and 17 (2.1 mg). Fraction 12 (9.0 g) was separated by MPLC using a gradient system of MeOH–H₂O (5:95–100:0, 50 mL/min) to obtain six fractions (Fr.12-1–Fr.12-6). Fraction 12-5 was chromatographed over a silica gel column using CHCl₃–MeOH (100:0–90:10, v/v) as solvent system, and then purified by semi-preparative HPLC using CH₂CN–H₂O (39:61, v/v) as mobile phase to give compound 9 (1.2 mg).

3.4. Structural Characterization

Triregelin A (1): yellow amorphous powder; [α]D²⁰ + 3.8 (c 0.50, MeOH); IR (KBr) νmax: 3444, 2925, 2854, 1732, 1601, 1455, 1377, 1260, 1167, 1086, 1013, 957, 893, 803, 756, 667 cm⁻¹; UV (MeOH) λmax (log ε) 260 (2.35) nm; CD (c 2.92 × 10⁻³ mol/L, MeOH) λmax (Δε) 225 (−1.22), 311 (+0.16); ¹H NMR (CDCl₃, 600 MHz) and ¹³C NMR (CDCl₃, 150 MHz) data, see Tables 1 and 2; HRESIMS m/z 343.1546 [M + H]+ (calcd for C₂₀H₂₃O₃, 343.1540).

Triregelin B (2): yellow amorphous powder; [α]D²⁰ + 75.5 (c 1.00, MeOH); IR (KBr) νmax: 3421, 2970, 2937, 2881, 1688, 1649, 1375, 1248, 1104, 1030, 977, 905, 798, 659, 599 cm⁻¹; UV (MeOH) λmax (log ε) 258 (3.17) nm; CD (c 1.46 × 10⁻³ mol/L, MeOH) λmax (Δε) 269 (7.24), 355 (+0.35), 476 (−0.54); ¹H NMR (CDCl₃, 600 MHz) and ¹³C NMR (CDCl₃, 150 MHz) data, see Tables 1 and 2; HRESIMS m/z 343.1560 [M − H]− (calcd for C₂₀H₂₃O₃, 343.1551).

Triregelin C (3): yellow, amorphous powder; [α]D²⁰ − 34.0 (c 1.00, MeOH); IR (KBr) νmax: 3437, 2966, 2936, 2876, 1700, 1650, 1463, 1430, 1383, 1294, 1235, 1165,1107, 1044, 974, 933, 900, 813, 741 cm⁻¹; UV (MeOH) λmax (log ε) 256 (3.42) nm; CD (c 1.45 × 10⁻³ mol/L, MeOH) λmax (Δε) 283 (+2.21), 359 (−0.25), 478 (−0.32); ¹H NMR (CDCl₃, 600 MHz) and ¹³C NMR (CDCl₃, 150 MHz) data, see Tables 1 and 2; HRESIMS m/z 347.1866 [M + H]⁺ (calcd for C₂₉H₂₈O₅, 347.1853).

Triregelin D (4): yellow, amorphous powder; [α]D²⁰ − 9.3 (c 1.00, MeOH); IR (KBr) νmax: 3435, 2964, 2932, 2877, 1688, 1652, 1606, 1462, 1426, 1383, 1293, 1234, 1192, 1085, 1039, 910, 856 cm⁻¹; UV (MeOH) λmax (log ε) 255 (3.19) nm; CD (c 1.39 × 10⁻³ mol/L, MeOH) λmax (Δε) 271 (+3.05), 357 (−0.22), 481 (−0.61); ¹H NMR (CDCl₃, 600 MHz) and ¹³C NMR (CDCl₃, 150 MHz) data, see Tables 1 and 2; HRESIMS m/z 361.2008 [M + H]⁺ (calcd for C₁₂H₂₀O₅, 361.2010).

Triregelin E (5): yellow, amorphous powder; [α]D²⁰ + 17.0 (c 0.50, MeOH); IR (KBr) νmax: 3455, 2962, 2930, 2874, 1744, 1713, 1650, 1604, 1464, 1384, 1294, 1233, 1104, 1042, 906, 802, 757, 666, 603 cm⁻¹; UV (MeOH) λmax (log ε) 257 (2.75) nm; CD (c 1.34 × 10⁻³ mol/L, MeOH) λmax (Δε) 261 (+3.35), 349 (+0.33), 474 (−0.30); ¹H NMR (CDCl₃, 600 MHz) and ¹³C NMR (CDCl₃, 150 MHz) data, see Tables 1 and 2; HRESIMS m/z 373.1998 [M + H]⁺ (calcd for C₂₂H₂₄O₅, 373.2010).

Triregelin F (6): yellow, amorphous powder; [α]D²⁰ +169.3 (c 1.00, MeOH); IR (KBr) νmax: 3437, 2964, 2872, 1626, 1424, 1250, 1160, 1115, 1059, 896, 815, 705 cm⁻¹; UV (MeOH) λmax (log ε) 223 (3.03), 270 (2.35) nm; CD (c 1.67 × 10⁻³ mol/L, MeOH) λmax (Δε) 209 (+5.51), 217 (sh) (+3.41), 265 (+1.02); ¹H NMR (CDCl₃, 600 MHz) and ¹³C NMR (CDCl₃, 150 MHz) data, see Tables 2 and 3; HRESIMS m/z 301.2162 [M + H]⁺ (calcd for C₁₀H₁₈O₄, 301.2162).

Triregelin G (7): yellow, amorphous powder; [α]D²⁰ − 46.9 (c 0.50, MeOH); IR (KBr) νmax: 3382, 2958, 2929, 2872, 1703, 1604, 1566, 1455, 1417, 1378, 1312, 1261, 1224, 1137, 1107, 1056, 802, 756, 667 cm⁻¹; UV (MeOH) λmax (log ε) 233 (3.23), 279 (2.37), 312 (2.45) nm; CD (c 1.45 × 10⁻³ mol/L, MeOH) λmax (Δε) 239 (−2.50), 286 (+1.08), 311 (−1.02), 392 (+0.31); ¹H NMR (CDCl₃, 600 MHz) and ¹³C NMR (CDCl₃, 150 MHz) data, see Tables 2 and 3; HRESIMS m/z 345.2055 [M + H]⁺ (calcd for C₂₁H₂₅O₄, 345.2060).

Triregelin H (8): yellow, amorphous powder; [α]D²⁰ + 56.4 (c 1.00, MeOH); IR (KBr) νmax: 3398, 2961, 2926, 2872, 1697, 1624, 1429, 1382, 1349, 1301, 1233, 1162, 1107, 1040, 964, 894, 801, 754 cm⁻¹; UV (MeOH) λmax (log ε) 238 (2.82), 270 (2.38) nm; CD (c 1.45 × 10⁻³ mol/L, MeOH) λmax (Δε) 233
(+2.77), 270 (−0.27), 310 (−1.08), 377 (+1.25); 1H NMR (CDCl$_3$, 600 MHz) and 13C NMR (CDCl$_3$, 150 MHz) data, see Tables 2 and 3; HRESIMS $m/z$ 347.1840 [M + H]$^+$ (calcd for C$_{29}$H$_{27}$O$_5$, 347.1853).

Triregelin I (9): white, amorphous powder; $[\alpha]_{D}^{22} + 29.4$ (c 0.50, MeOH); IR (KBr) $\nu_{max}$: 3396, 2959, 2924, 2854, 1714, 1592, 1428, 1348, 1260, 1168, 1114, 1028, 970, 800, 755, 709 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 235 (3.32), 270 (3.22), 380 (2.95) nm; CD (c 1.44 × 10$^{-3}$ mol/L, MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 206 (+2.23), 235 (+0.88), 271 (−0.65), 311 (−0.61), 375 (+0.96); 1H NMR (CDCl$_3$, 600 MHz) and 13C NMR (CDCl$_3$, 150 MHz) data, see Tables 2 and 3; HRESIMS $m/z$ 349.2005 [M + H]$^+$ (calcd for C$_{29}$H$_{27}$O$_5$, 349.2010).

Triregelin J (10): white, amorphous powder; $[\alpha]_{D}^{22} + 1.4$ (c 1.00, MeOH); IR (KBr) $\nu_{max}$: 3364, 2962, 2937, 2870, 1622, 1558, 1455, 1427, 1381, 1347, 1251, 1212, 1160, 1113, 1080, 1037, 981, 914, 821, 757, 711, 663, 582 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 216 (3.40), 266 (2.33), 343 (2.42) nm; CD (c 1.51 × 10$^{-3}$ mol/L, MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 218 (+1.82), 230 (+1.34), 266 (−3.14), 333 (+1.23), 346 (+1.71); 1H NMR (CDCl$_3$, 600 MHz) and 13C NMR (CDCl$_3$, 150 MHz) data, see Tables 2 and 3; HRESIMS $m/z$ 333.2053 [M + H]$^+$ (calcd for C$_{29}$H$_{29}$O$_5$, 333.2060).

Triregelin K (11): white, amorphous powder; $[\alpha]_{D}^{22} + 28.1$ (c 0.25, MeOH); IR (KBr) $\nu_{max}$: 3396, 3210, 2926, 2865, 1737, 1607, 1581, 1441, 1412, 1373, 1331, 1308, 1268, 1206, 1101, 1041, 942, 926, 857, 800, 756, 695, 660 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 227 (2.82), 283 (2.36) nm; CD (c 1.51 × 10$^{-3}$ mol/L, MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 228 (+0.97); 1H NMR (pyridine-$d_5$, 600 MHz) and 13C NMR (pyridine-$d_5$, 150 MHz) data, see Tables 2 and 3; HRESIMS $m/z$ 333.2426 [M + H]$^+$ (calcd for C$_{29}$H$_{33}$O$_5$, 333.2424).

Triregelin L (12): white, amorphous powder; $[\alpha]_{D}^{22} + 50.86$ (c 0.50, MeOH); IR (KBr) $\nu_{max}$: 3475, 3072, 2961, 2924, 2867, 1710, 1655, 1607, 1510, 1445, 1415, 1369, 1261, 1089, 1028, 882, 801, 701, 665 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 203 (3.15) nm; CD (c 1.66 × 10$^{-1}$ mol/L, MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 203 (−24.65), 295 (+6.40); 1H NMR (CDCl$_3$, 600 MHz) and 13C NMR (CDCl$_3$, 150 MHz) data, see Tables 1 and 2; HRESIMS $m/z$ 303.2322 [M + H]$^+$ (calcd for C$_{29}$H$_{31}$O$_2$, 303.2319).

3.5. Calculation Methods of Electronic Circular Dichroism (ECD) Spectra

The Gaussian 09 software package [32] was used to conduct all of the ECD calculations. The molecular geometries of molecules were firstly optimized at the level of B3LYP/6-31G (d, p) and the output geometries were subsequently employed to perform ECD calculations using time-dependent density functional theory (TDDFT) with the method of B3LYP/DGDZVP [20,21] since this method usually offers desirable outcomes [33]. The model of polarizable continuum was utilized to simulate the solvation effect in the calculations of circular dichroism. The experimental condition was simulated by using methanol as the solvent. The absolute configurations of all compounds were defined by comparing the calculated ECD curves with the experimental spectra.

3.6. Cytotoxicity of Diterpenes against Three Cancer Cell Lines

The A2780 (ovarian carcinoma) cell line was obtained from the KeyGEN biotech (Nanjing, China). HepG2 (hepatocellular carcinoma) and MCF-7 (human breast cancer) cell lines were purchased from the American Type Culture Collection. All of the cell lines were cultured in Dulbecco’s modified Eagle medium (DMEM) (Invitrogen) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Invitrogen), 100 U/mL penicillin, and 100 µg/mL streptomycin (Invitrogen) in a humidified atmosphere of 5% CO$_2$/95% air at 37 °C. Briefly, cells were seeded in 96-well plates in triplicate at a density of 2 × 10$^4$ cells/well (100 µL) and cultured at 37 °C in a 5% CO$_2$ humidified atmosphere for 24 h. Then, the cells were treated with fresh culture medium containing various concentrations of tested compounds and incubated at 37 °C under a humidified atmosphere of 5% CO$_2$/95% air for another 72 h. After that, the supernatant in each well was discarded and the cells were washed by phosphate-buffered saline (PBS) to avoid the possible effect of culture medium and tested compounds on the following MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Subsequently, cells were incubated for 4 h at 37 °C in culture medium containing a final concentration of 0.5 mg/mL MTT (100 µL). The formed formazan crystals were dissolved in DMSO (100 µL) after removing the supernatant in each well. A microplate reader (Infinite 200 PRO,
Tecan, Männedorf, Switzerland) was employed to determine the absorbance of each well at 570 nm. GraphPad Prism 6 software (Prism 6.0, GraphPad Software, Inc., La Jolla, CA, USA) was used to calculate the IC\textsubscript{50} values (concentration that suppresses 50% of cell growth) of all tested compounds. All assays were performed in triplicate in three independent experiments. Data was expressed as mean ± SD (n = 3).

4. Conclusions

To sum up, 23 diterpenoids were isolated from the Chinese herbal medicine \textit{T. regelii}, including eleven new abietane, and one new kaurane, diterpenes. Importantly, triregelin A (1) represents the first abietane diterpene bearing an 18→1 lactone ring. Triregelin I (9) exhibited significant cytotoxic effects on A2780 and HepG2 cancer cells with IC\textsubscript{50} values of 5.88 \(\mu\text{M}\) and 11.74 \(\mu\text{M}\), respectively, and was found inactive against MCF-7 cancer cells. Triregelin K (11) displayed a weak cytotoxic effect on MCF-7 cell with an IC\textsubscript{50} value of 26.70 \(\mu\text{M}\).

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/1422-0067/18/1/147/s1.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| HRESIMS      | High resolution electrospray ionization mass spectrometry |
| CD           | Circular dichroism |
| UV           | Ultraviolet visible |
| IR           | Infrared |
| NMR          | Nuclear magnetic resonance |
| DEPT         | Distortionless enhancement by polarization transfer |
| HSQC         | Heteronuclear single quantum coherence |
| HMBC         | Heteronuclear multiple bond correlation |
| \textsuperscript{1}H–\textsuperscript{1}H COSY | Proton–proton correlation spectroscopy |
| NOESY        | Nuclear Overhauser effect spectroscopy |

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