HETEROCYCLES, Vol. 100, No. 4, 2020, pp. 559 - 567. © 2020 The Japan Institute of Heterocyclic Chemistry
Received, 29th January, 2020, Accepted, 28th February, 2020, Published online, 11th March, 2020
DOI: 10.3987/COM-20-14223

# ABSOLUTE CONFIGURATION OF THE BUTENOLIDE OF ABIETANE

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**Abstract** – 17-Hydroxyjolkinolide A-B (**1**-**2**) and jolkinolide A-B (**3**-**4**) are four *ent*-13(15)-abietane-16,12-olide type diterpenoids isolated from the roots of *Euphorbia fischeriana* Steud. The absolute configurations of **1** and **2** were established by X-ray crystallographic analysis of their mono-bromobenzoate derivatives. On basis of our research, the absolute configuration of the butenolide of abietane can be determined by ECD spectra. The potential antituberculosis effects of these diterpenoids were evaluated using a *Mycobacterium smegmatis* model. The most potent compound according to the *in vitro* bioassay used was 17-hydroxyjolkinolide B (**2**) (MIC 1.8 $\mu$g/mL).

## INTRODUCTION

*Euphorbia fischeriana* Steud is a perennial herbaceous plant belonging to the family Euphorbiaceae, which is primarily distributed in northeastern mainland China.[1] The dried plant roots of *E. fischeriana* named "lang-du" in traditional Chinese medicine, have been used as a remedy for the treatment of ailments, including edema, ascites and cancer.[2–4] The main components of *E. fischeriana* are diterpenoids, triterpenes, steroids, aromatic compounds and tannins.[3] Among them, diterpenoids are the primary bioactive constituents of this plant. Diterpenoids are a focus of natural product drug discovery because of their great structural diversity and pronounced biological activities.[5] A number of abietane diterpenoids including jolkinolides A and B, and 17-hydroxyjolkinolides A and B have been isolated.

The structural elucidation of complex natural products has been a great challenge for organic chemists, especially for the assignment of the absolute configuration. In current natural product research,

single-crystal X-ray diffraction and chiroptical methods (such as CD, ECD, etc) are the most important and popular tools for determining the absolute configuration of novel natural products. As part of our research on bioactive diterpenoids from euphorbiaceous plants, we have investigated the dried roots of *E. fischeriana* and 17-hydroxyjolkinolides A (**1**) and B (**2**), along with jolkinolides A (**3**) and B (**4**) were isolated, which belonged to *ent*-13(15)-abietane-16,12-olide type diterpenoids. The absolute configurations of 17-hyodroxyjolkinolide A-B (**1**-**2**) were established by single-crystal X-ray diffraction of their mono-bromobenzoate derivatives. The absolute configuration of the butenolide of abietane is very difficult to determine before this. In this paper, we study the relationship between the ECD speactra and the absolute configuration of the butenolide of abietane on the basis of the X-ray diffraction result, which make it more accurate to determine the absolute configuration using ECD. Furthermore, the inhibitory effects of the isolated compounds were evaluated against *Mycobacterium smegmatis*.

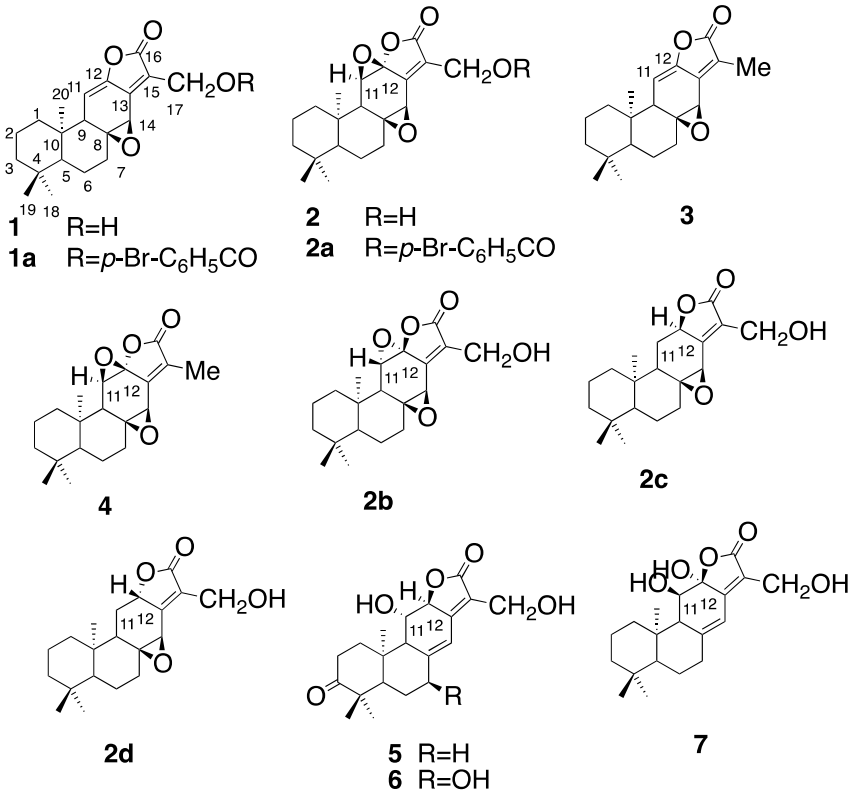


**Figure 1.** Structures of Compound **1**-**7**

## RESULTS AND DISCUSSION

Compound **1** was obtained as colorless needles with $[\alpha]^{25}_{D}$ 96.7 (c 0.17, $CHCl_3$). The molecular formula, $C_{20}H_{26}O_4$, was determined by HRESIMS (*m/z* 353.1834 $[M+Na]^+$ (Calcd. for $C_{20}H_{26}O_4Na$, *m/z* 353.1831)). It was determined as 17-hydroxyjolkinolide A based on the same spectroscopic and physical properties as those previously reported.[3]

The 17-*O*-*p*-bromobenzoyl ester of **1** (**1a**) was synthesized for the absolute configuration determination by single-crystal X-ray diffraction. For the crystal structure of **1a**, it is the hydrated form with 0.5 molecule water per asymmetric unit. Both rings A and B are in chair conformations, whereas ring C adopts a flattened boat conformation, and ring D is intrinsically planar. The torsion angle of C16-C15-C17-O6 is 69.9º(6), the molecule then has a relatively liner shape. The absolute configuration of **1a** was determined by anomalous scattering of X-ray diffraction, the five stereogenic centers C5/C8/C9/C10/C14 were in *R*/*S*/*S*/*R*/*R* configurations, respectively (Figure 2).

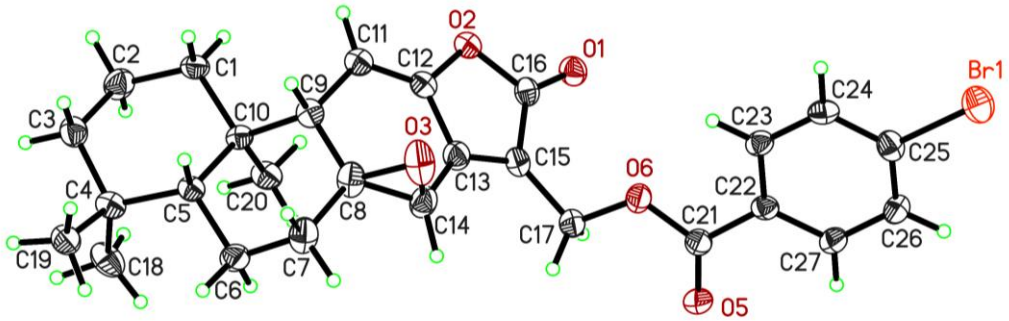


**Figure 2.** X-Ray structural diagram of **1a**

Compound **2** was obtained as colorless needles with $[\alpha]^{25}_{D}$ 162.5 (c 0.46, $CHCl_3$). It possesses the molecular formula of $C_{20}H_{26}O_5$ as deduced from its HRESIMS (*m/z* 369.1783 $[M+Na]^+$ (Calcd. for $C_{20}H_{26}O_5Na$, *m/z* 369.1780)). It was identified as 17-hydroxyjolkinolide B by comparing its spectroscopic data with those reported.[3] The 17-*O*-*p*-bromobenzoyl ester of **2** (**2a**) was also prepared for the absolute configuration determination by single-crystal X-ray diffraction and a perspective ORTEP plot is shown in Figure 3.

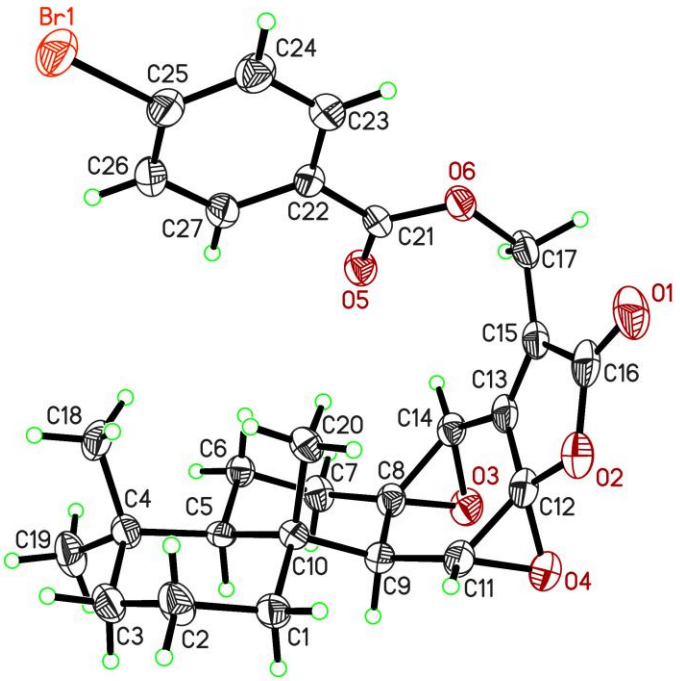


**Figure 3.** X-Ray structural diagram of **2a**

In the crystal structure of **2a**, both rings A and B are in chair conformations, whereas ring C adopts a flattened boat conformation, while ring D is intrinsically planar, this geometry is very similar to **1a**. In contrast to the linear shape of **1a**, the torsion angle of C16-C15-C17-O6 in **2a** is -73.5º(5), the molecule becomes U-shape instead. The absolute configuration of **2a**, again by anomalous scattering of X-ray diffraction, has been determined and the chiral centers are in (5*R*, 8*S*, 9*R*, 10*R*, 11*R*, 12*R*, 14*R*).

When the absolute configuration determination was finished by the X-ray analysis, the experimental CD and calculated ECD spectra of compound **2** were tested. It had been reported that the α,β-unsaturated γ-lactones usually showed Cotton effects associated with the π-π* transition in the region 205-235 nm and the n-π* transition in the region 245-274 nm.[6-7] Wang et al. reported that the absolute configurations of butenolides in *ent*-abietanes were established by ECD experiments,[1] but they had never been confirmed by X-ray analysis.

The CD spectrum of **2** (11β, 12β-epoxy, 12*R*) showed a negative Cotton effect at 208 nm and a positive Cotton effect at 260 nm. The calculated ECD spectrum of **2** showed a negative Cotton effect at 218 nm (π-π* transition) and a positive Cotton effect at 252 nm (n-π* transition). The calculated ECD spectrum of **2** matched very well with experimental spectrum. The calculated ECD curve of the isomer of **2** (**2b**, 11α, 12α-epoxy, 12*S*) showed the Cotton effects at 211 nm (positive, π-π*) and 275 nm (negative, n-π*) that was opposite to **2** (Figure 4, Table 1). The β-carbon atom of lactone in compound **2** was below the

-CO-O- plane and it showed the negative sign associated with the π-π* transition. This result was consistent with Beecham rule.[6] Thus it confirmed that the absolute configuration of C-12 in compound **2** could be assigned by the ECD method.

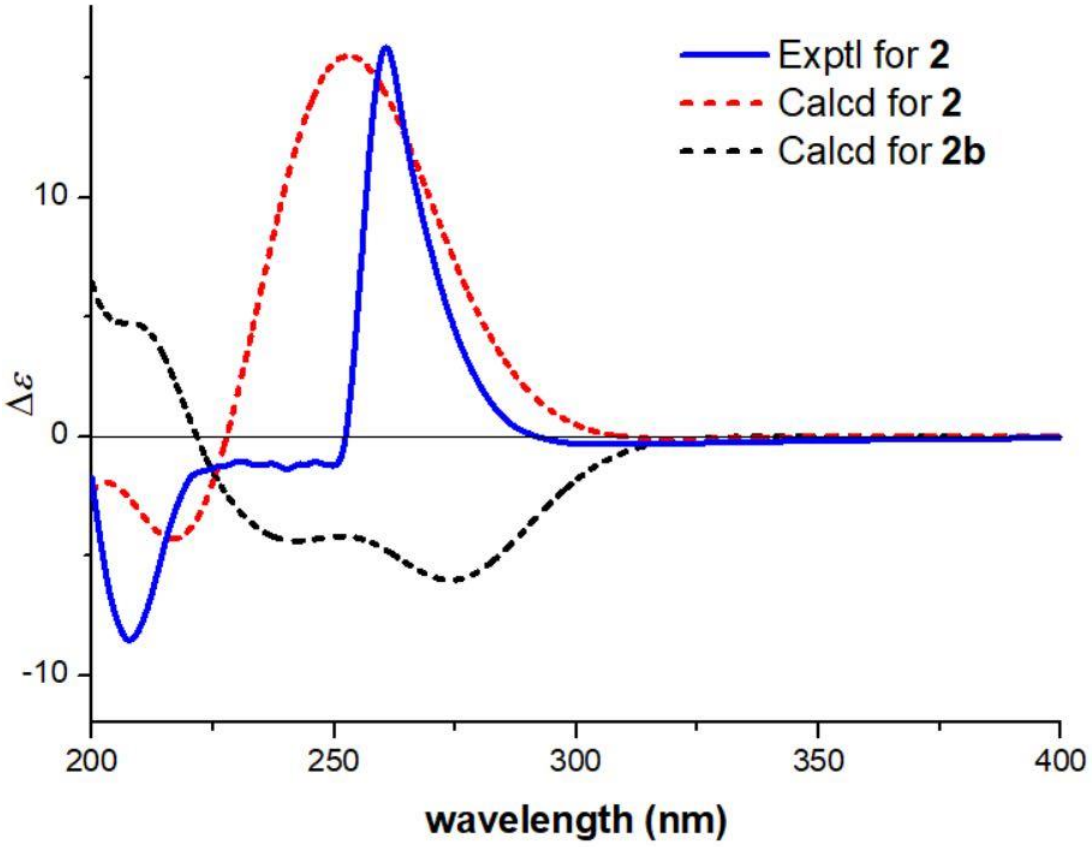


**Figure 4.** Measured CD spectra of **2** (blue line) and the calculated ECD spectra of **2** (red line) and **2b** (black line)

The abietanes that had no substituent at C-12 of the butenolide as compounds **2c** (12α-H) and **2d (**12β-H**)** were found most common in the nature. In order to further investigate the absolute configuration of the butenolide of abietane, we calculated the ECD curves of compound **2c** and its isomer **2d**. Compound **2c** (12α-H, 12*R*) showed the negative Cotton effect at 211nm (π-π*) and positive Cotton effect at 244 nm (n-π*). Compound **2d** (12β-H, 12*S*) showed the positive Cotton effect at 211 nm (π-π*) and negative Cotton effect at 234 nm (n-π*). The CD and ECD data listed in Table 1 showed that the π-π* Cotton effect of the butenolide was reliable and unambiguous for both the sign and wave number. But we also found that the wave number of the n-π* Cotton effect of the butenolide was easily influenced by the different substitute group at C-12 (Table 1). Therefore the π-π* CD is better than the n-π* CD for the absolute configuration determination of the butenolide in abietane.

**Table 1.** The CD and ECD data for the butenolide of abietane

| Compound | C-12 | | CD or ECD | λ nm (Δε) | |
|---|---|---|---|---|---|
| | AC[a] | RC[b] | | π-π* | n-π* |
| 17-hydroxyjolkinolide B (**Expl.2**) | *R* | β-O- | X-Ray; CD | 208 (-8.8) | 260 (+16.5) |
| 17-hydroxyjolkinolide B (**2**) | *R* | β-O- | ECD | 218 (-4.2) | 252 (+16) |
| 17-hydroxy-11α,12α;8β,14β-diepoxy-13(15)-abietane-16,12β-olide (**2b**) | *S* | α-O- | ECD | 211 (+4.7) | 275 (-6.2) |
| 17-hydroxy-8β,14β-epoxy-13(15)-abietane-16,12β-olide (**2c**) | *R* | α-H | ECD | 211 (-40) | 244 (+32) |
| 17-hydroxy-8β,14β-epoxy-13(15)-abietane-16,12α-olide (**2d**) | *S* | β-H | ECD | 211 (+38) | 234 (-36) |
| 11α,17-dihydroxyhelioscopinolide E (**5**) [1] | *S* | α-H | ECD | 236.5 (-4.3) | 277 (+14) |
| 6β,11α,17-trihydroxyhelioscopinolide E (**6**) [1] | *S* | α-H | ECD | 234.5 (-1.43) | 275 (+7.2) |
| 7-deoxylangduin B (**7**) [1] | *S* | α-OH | ECD | 248.5 (+1.83) | 287.5 (-3.13) |

[a] Absolute Configuration; [b] Relative Configuration

Comparing the ECD spectra of the abietanes containing oxygen groups on C-12 of butenolides and the abietanes that had non-substituent on C-12 of butenolides, we found the conclusion was consistent with Beecham [6] and Uchida rule.[7]

In conclusion, for the abietanes that had non-substituent group on C-11 and C-12 of butenolide, if it exhibited negative π-π* and positive n-π* Cotton effect, the configuration of C-12 of butenolide could be designated as *R* (12α-H, **2c**) and if it showed positive π-π* and negative n-π* Cotton effect, the configuration of C-12 could be specified as *S* (12β-H, **2d**). For the abietanes containing oxygen groups on C-11 and C-12 of butenolide, if it showed negative π-π* and positive n-π* Cotton effect, the configuration of C-12 of butenolide could be designated as *R* (12β-O-, **2**) and if it showed positive π-π* and negative n-π* Cotton effect, the configuration of C-12 could be designated as *S* (12α-O-, **2b**, **7**). For the abietane that had oxygen group on C-11 and non-substituent on C-12 of butenolide (**5**, **6**), if it showed negative π-π* and positive n-π* Cotton effect, the H-12 was in an α configuration and the C-12 could be specified

as *S*. So the absolute configuration of butenolide of abietane (C-12) can be determined by ECD spectra on the basis of our research.

Compounds **3** and **4** were identified as jolkinolide A and B based on the same spectroscopic and physical properties as those previously reported.[3] The CD curve of compound **4** was tested and it also showed a negative Cotton effect at 208 nm ($\pi$-$\pi^*$ transition) and a positive Cotton effect at 260 nm (n-$\pi^*$ transition). According to our previous research conclusions, we can deduce the absolute configuration of C-12 of compound **4** as *R*. On the basis of the relative configuration determined before, the absolute configuration of **4** could be identified as (5*R*, 8*S*, 9*R*, 10*R*, 11*R*, 12*R*, 14*R*).

The MIC values of the isolated compounds against *M. smegmatis* were evaluated. However, only 17-hydroxyjolkinolide B (**2**) was found to be an active compound and exhibit an MIC value of 1.8 $\mu$g/mL.

## EXPERIMENTAL

**General Experimental Procedures.** Melting points were measured on a Kofler micro-melting apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 16 PC FT-IR spectrometer. 1D and 2D NMR spectra were run on Bruker DRX-500 and JEOL JNM-EX 400 spectrometers. HRESIMS were obtained on a PE Biosystems Mariner System 5140 LC-MS spectrometer. Silica gel (Merck, Kieselgel 60, 230-400 mesh) was used for column chromatography.

**Plant Materials.** The dried roots of *Euphorbia fischeriana* Steud were collected from Chifeng, Neimeng Province, People's Republic of China in October 2006 and identified by Prof. Minjian Qin (Department of Natural Medicinal Resources, China Pharmaceutical University, Nanjing, China). A voucher specimen (No.35-72-56-6) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

**Extraction and Isolation.** Dried roots of *Euphorbia fischeriana* Steud (5 kg) were crushed and extracted with 95% EtOH (2 h each) under reflux three times. The EtOH extracts, after removal of the solvent, were suspended in $H_2O$ and then extracted with EtOAc (three times) successively to yield fraction of 1300 g. The EtOAc fraction was subjected to silica gel column chromatography eluting with a gradient of petroleum ether-EtOAc (petroleum ether, 9:1, 8:2, 7:3, 6:4, 1:1, EtOAc) to yield fractions 1-13. Fraction 5 (1.4 g) and fractions 6-7 (1.2 g) were recrystallized to obtain **3** (400 mg) and **4** (250 mg). Fraction 9 (23 g) was separated by repeated silica gel column chromatography to give **1** (260 mg). Fraction 10 (35 g) was subjected to repeated silica gel column chromatography to yield **2** (130 mg).

**17-Bromobenzoate of 17-hydroxyjolkinolide A (1a).** A sample of **1** (110 mg) was dissolved in $CH_2Cl_2$ (5 mL) in a 50 mL round-bottomed flask and combined with DMAP (70 mg) and 4-bromobenzoyl chloride (110 mg). The reaction vessel was sealed and the contents were stirred at room temperature. TLC was monitoring until the reaction was over. Then the reaction solution was poured into saturated sodium hydrogen carbonate aqueous solution and extracted with $CH_2Cl_2$. The extract was dried with magnesium sulfate, and evaporated to afford a residue. The residue was subjected to silica gel chromatography, eluted petroleum ether-acetone (100:8) to afford **1a** (160 mg, yield 93.5%).

**17-Bromobenzoate of 17-hydroxyjolkinolide B (2a).** A sample of **2** (67 mg) was dissolved in $CH_2Cl_2$ (3 mL) in a 25 mL round-bottomed flask and combined with DMAP (40 mg) and 4-bromobenzoyl chloride (70 mg). The reaction vessel was sealed and the contents were stirred at room temperature. TLC was monitoring until the reaction was over. Then the reaction solution was poured into saturated sodium hydrogen carbonate aqueous solution and extracted with $CH_2Cl_2$. The extract was dried with magnesium sulfate, and evaporated to afford a residue. The residue was subjected to silica gel chromatography, eluted petroleum ether-acetone (10:1) to afford **2a** (98 mg, yield 95.6%).

**X-Ray Structure Determinations of 1a and 2a.** Colorless elongated block-shaped crystals of **1a** were slowly crystallized from a $CHCl_3$-MeOH solution at room temperature. Same experimental protocols were applied to give the colorless needle-shaped crystals of **2a**. Selected crystals were mount on MiTeGen$^{TM}$ loops, and the diffraction intensity data were collected by a Rigaku SuperNova, Dual, Atlas diffractometer with Cu-K$\alpha$ source ($\lambda$=1.54178Å) at 173.00(10) K. Using Olex2, [8] the structure was solved with the ShelXS[9] structure solution program using Direct Methods and refined with the ShelXL[10] refinement package using Least Squares minimization. All non-hydrogen atoms were refined anisotropically by full-matrix least squares with a riding model for the hydrogen atoms.

**Crystal structure determination of 1a.** Crystal Data for $C_{27}H_{29.5}BrO_{5.25}$ (M =517.91 g/mol): monoclinic, space group C2 (no. 5), a = 22.7527(9) Å, b = 5.92651(20) Å, c = 18.8568(8) Å, $\beta$ = 111.017(5)°, V = 2373.58(17) Å$^3$, Z = 4, T = 173.00(10) K, $\mu$(CuK$\alpha$) = 2.670 mm$^{-1}$, Dcalc = 1.449 g/cm$^3$, 6924 reflections measured (8.034° $\leq 2\Theta \leq$ 134.988°), 4209 unique ($R_{int}$ = 0.0322, $R_{sigma}$ = 0.0475) which were used in all calculations. The final $R_1$ was 0.0364 (I > 2$\sigma$(I)) and $wR_2$ was 0.0875 (all data). (CCDC number 753360)

**Crystal structure determination of 2a.** for $C_{27}H_{29}BrO_6$ (M =529.41 g/mol): orthorhombic, space group $P2_12_12_1$ (no. 19), a = 7.12487(19) Å, b = 18.2574(5) Å, c = 18.4992(4) Å, V = 2406.41(10) Å$^3$, Z = 4, T = 173.2(3) K, $\mu$(CuK$\alpha$) = 2.671 mm$^{-1}$, Dcalc = 1.461 g/cm$^3$, 13169 reflections measured (6.802° $\leq 2\Theta \leq$

134.946°), 4341 unique ($R_{int}$ = 0.0536, $R_{sigma}$ = 0.0499) which were used in all calculations. The final $R_1$ was 0.0402 ($I > 2\sigma(I)$) and $wR_2$ was 0.0925 (all data). (CCDC number 753361)

**Inhibitory Effects on *Mycobacterium smegmatis.*** The inhibitory effects of different extracts and isolated diterpenoids against *M. smegmatis* were conducted using an Alamar blue cell viability assay (Thermo Fisher Scientific Inc.) in 96-well microplates, as described previously.[11] The test materials were dissolved in DMSO at an initial concentration at 0.4 mg/mL. The positive control antimicrobial agent, kanamycin, was used at a concentration of 0.1 mg/mL. The *M. smegmatis* strain was preincubated for 48 h and incubated for an additional 24 h at 37 °C after the addition of the samples. Then, cell viability was determined by absorbance measurement at 590 nm.

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