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3,4-*seco*-Diterpenoids from *Trigonostemon flavidus*

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ABSTRACT

Phytochemical investigation on the stems of *Trigonostemon flavidus* resulted in the isolation of five new 3,4-*seco*-diterpenoids, trigoflavidones A–E (**1**–**5**), structurally related to the main co-occurring known 3,4-*seco*-sonderianic acid (**6**) and 3,4-*seco*-sonderianol (**7**). Compound **4** possesses new 3,4-*seco* rearranged *ent*-pimarane skeletal type, characteristic of a vinyl group at C-8, while **5** features a unique five-membered ring (C_1) fused with a cyclopropane ring (C_2). The structures of the new compounds were established by a combination of spectroscopic data and computational methods. Compounds **1**–**7** were tested for their cytotoxicities on HL-60, SMMC-7721, A-549, MCF-7, and SW480 human tumor cell lines.

1. Introduction

Trigonostemon plants (Euphorbiaceae), distributed widely in tropical and subtropical areas of Asia,[1] have been used in ethnomedicine as an expectorant and for the treatment of asthma and diarrhea.[2] So far, various structurally interesting natural products such as alkaloids,[3–6] modified daphnane diterpenoids,[7–12] degraded diterpenoids,[12–16] and 3,4-*seco*-cleistantane diterpenoids[12,14,17] have been isolated from this genus. Especially, these diterpenoids exhibited a wide range of biological activities, such as insecticidal,[7,18] antimicrobial,[14,16] cytotoxic,[8,19] and antiviral[9,11,20] properties. Previous phytochemical investigation on *Trigonostemon flavidus* Gagnepain (also known as *Trigonostemon heterophyllus* Merrill)[1] resulted in the isolation of the major classes of chemical constituents of this genus, such as daphnane diterpenoids,[12] degraded diterpenoids,[12,16] and 3,4-*seco*-diterpenoids.[17] As a part of our ongoing research on this plant,[16] seven 3,4-*seco*-diterpenoids (Fig. 1) including five new ones, trigoflavidones A–E (**1**–**5**), were isolated from the stems of *T. flavidus* collected from Hainan Province of China. Trigoflavidones D (**4**) and E (**5**) are new skeletal types of 3,4-*seco*-diterpenoids. Compound **4** possesses new 3,4-*seco* rearranged *ent*-pimarane skeletal type, characteristic of a vinyl group at C-8, while **5** features a unique five-membered ring (C_1) fused with a cyclopropane ring (C_2). The absolute configuration of **1**–**5** was determined by comparing their theoretical calculation of electronic circular dichroism (ECD) spectra and optical rotation (OR) values with the corresponding experimental data as well as on the basis of their biogenetic relationships. In this paper, we report the isolation and structural elucidation of trigoflavidones A–E (**1**–**5**), as well as their cytotoxicities against five human tumor cell lines.

2. Results and discussion

Trigoflavidone A (**1**) exhibited a pseudomolecular ion peak (HRESIMS, positive-ion mode) at *m*/*z* 351.1932 $[M+Na]^+$ (calcd for $C_{21}H_{28}O_3Na$, 351.1936), allowing the determination of a molecular formula $C_{21}H_{28}O_3$ with eight degrees of unsaturation. The ^1H NMR spectrum of **1** displayed signals for a vinyl moiety [δ_H 5.75 (1H, dd, J=17.5 and 10.2 Hz), 5.04 (1H, d, J=17.5 Hz), and 5.01 (1H, d, J=10.2 Hz)], an isopropenyl group [δ_H 4.82 (1H, s), 4.65 (1H, s), and 1.66 (3H, s)], two other olefinic protons [δ_H 5.96 (1H, s) and 5.90 (1H, s)], and three other singlet methyls [δ_H 3.61 (OMe), 1.27, and 1.12], which showed correlations in the HSQC spectrum with the olefinic carbon signals at δ_C 139.5, 114.3, 114.0, 145.9, 139.6, 129.6, 121.0, and 162.1, the methoxyl carbon signal at δ_C 51.6, and the methyl carbon signals at δ_C 22.3, 23.8, and 24.6. In addition, nine carbons including four methylenes, one methine, four quaternary

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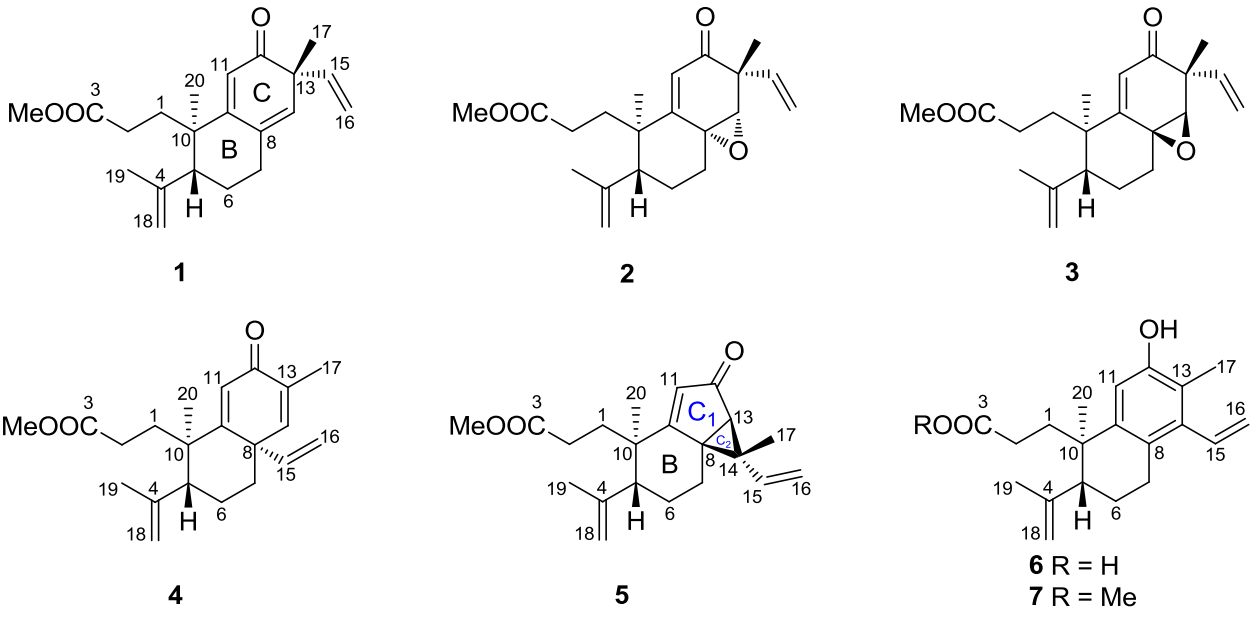


Fig. 1. Chemical structures of compounds **1**–**7**.

carbons (one ester carbonyl, one conjugated carbonyl, and two high-field ones) (Table 2) were observed in the ^{13}C NMR and DEPT spectra of **1**. According to aforementioned data, to satisfy the degrees of unsaturation, compound **1** was suggested to be a dicyclic diterpenoid, structurally similar to the coexisting major diterpenes, 3,4-*seco*-sonderianic acid (**6**)[21] and 3,4-*seco*-sonderianol (**7**),[21] by comparison of their MS and NMR data. The main differences were the presence of a highly conjugated ketone carbonyl, two singlet olefin protons, and other one high-field quaternary carbon in **1** according to its 1D NMR, which maybe due to the location of the vinyl moiety. Key HMBC correlations of H-11 to C-8 and C-13, H_2-16 to C-13, H_3-17 to C-12, C-14, and C-15, and H-14 to C-9, C-12, C-15, and C-17, established ring C with the assignments of the high-field quaternary carbon (δ_C 49.3) and CH_3-17 and the vinyl moiety at C-13, as well as the adjacent carbonyl and olefinic carbons. Other parts of **1** were determined to be the same as **7** by analysis of the $^1H-^1H$ COSY and HMBC correlations (Fig. 1). Moreover, the structure of trigoflavidone A was further confirmed by comparison its NMR data with that of trigonoheterene,[17] a 3,4-*seco*-*ent*-pimarane diterpenoid, isolated from this plant.

The key ROESY cross-peaks (Fig. 1) confirmed the 1,3-diaxial *syn* relationships of H-5 and H-7β and of H_3-20 and H-6α in **1**. Moreover, the configuration of the vinyl group was deduced to be α-orientation on the basis of the close biogenetic relationship between **1** and **4**, which was explained below and shown in Scheme 1.

Table 1
^1H NMR data of **1**–**5** in $CDCl_3$ (δ in ppm)

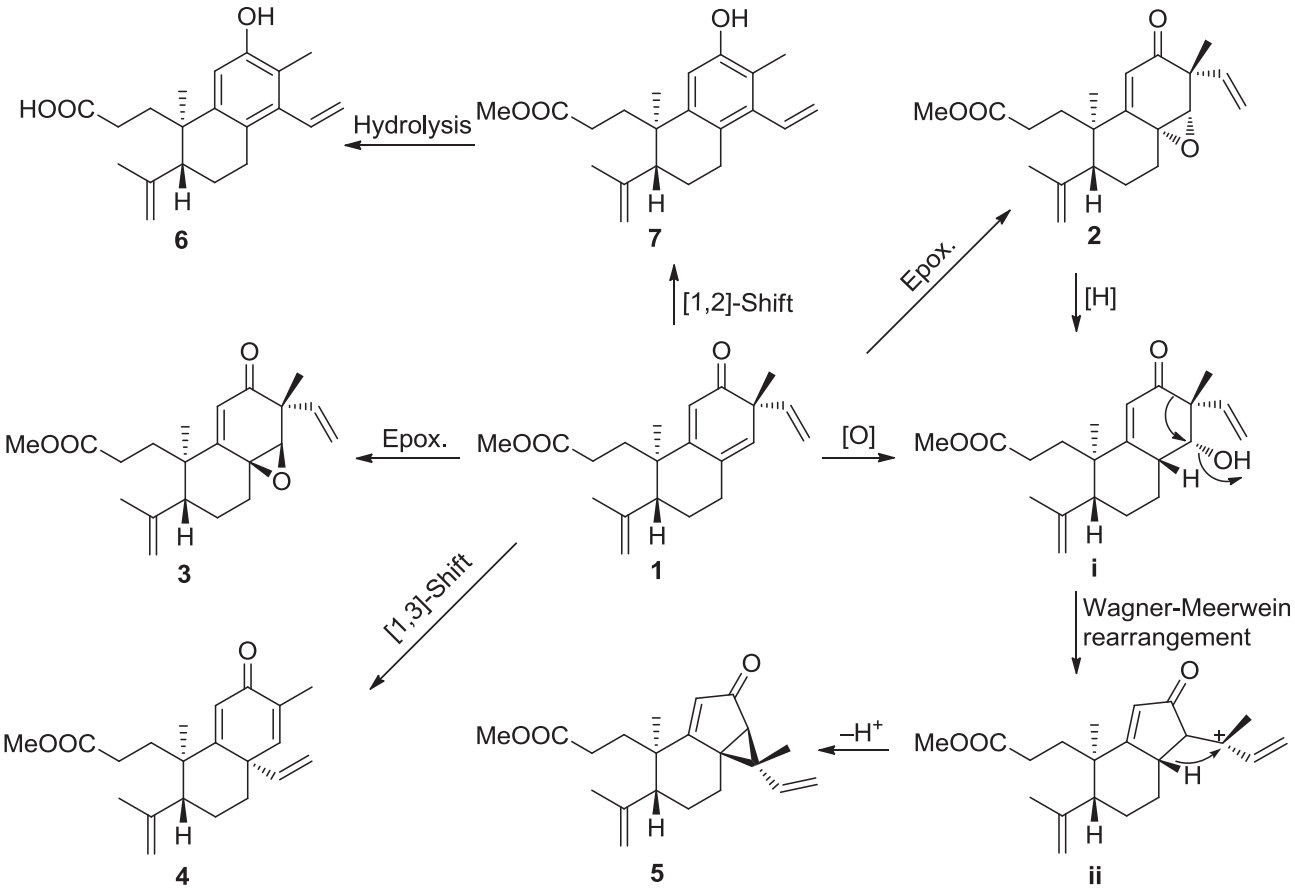
Position	**1**[a]	**2**[b]	**3**[a]	**4**[b]	**5**[b]
	δ_H, multi (J in Hz)	δ_H, multi (J in Hz)	δ_H, multi (J in Hz)	δ_H, multi (J in Hz)	δ_H, multi (J in Hz)
1a	1.87, m	1.99, m	1.96, m	1.85, m	1.74, m
1b	1.99, m		2.05, m	2.06, m	1.95, m
2a	2.23, t (8.3)	2.20, m	2.18–2.24, m	2.38–2.44, m	2.23, m
2b		2.37, m	2.18–2.24, m	2.38–2.44, m	2.34, m
5	2.31, t (6.4)	2.32, dd (11.5, 3.3)	2.28, m	2.11, m	2.25, t (4.0)
6α	1.71, m	2.13, m	1.58, m	2.13, m	1.52, m
6β	1.94, m	1.68, m	2.29, m	1.54, m	1.93, m
7α	2.50–2.62, m	2.17, m	2.46, m	2.27, m	2.15, td (13.3, 4.5)
7β	2.50–2.62, m	1.60, m	1.56, m	1.45, m	1.88, m
11	5.90, s	5.89, s	5.85, s	6.18, s	5.73, s
14	5.96, s	3.16, s	3.18, s	6.18, s	2.06, s, H-13
15	5.75, dd (17.5, 10.2)	6.12, dd (17.7, 10.7)	5.76, dd (17.6, 10.7)	5.42, dd (17.4, 10.5)	5.73, dd (17.3, 10.8)
16a	5.017, d (17.5)	5.29, d (10.7)	5.11, d (17.6)	5.19, d (17.4)	5.16, d (17.3)
16b	5.024, d (10.2)	5.31, d (17.7)	5.21, d (10.7)	5.21, d (10.5)	5.20, d (10.8)
17	1.27, s	1.29, s	1.43, s	1.85, s	1.22, s
18a	4.65, s	4.78, s	4.63, s	4.75, s	4.64, s
18b	4.82, s	4.96, s	4.95, s	4.93, s	4.92, s
19	1.66, s	1.80, s	1.76, s	1.76, s	1.72, s
20	1.12, s	1.22, s	1.16, s	1.14, s	1.15, s
3-OCH_3	3.61, s	3.65, s	3.66, s	3.67, s	3.68, s

[a] Measured at 400 MHz.
[b] Measured at 600 MHz.

Table 2
^{13}C NMR data of 1–5 in $CDCl_3$ (δ in ppm)

Position	**1**[a]	**2**[b]	**3**[a]	**4**[b]	**5**[b]
1	35.1	32.0	36.0	33.3	33.5
2	29.0	29.1	28.9	29.0	29.4
3	173.6	173.7	173.7	174.1	174.0
4	145.9	145.8	144.4	145.8	144.7
5	48.2	47.3	49.6	51.1	50.6
6	23.5	24.1	20.9	23.9	23.8
7	27.3	32.4	26.9	32.6	22.2
8	129.6	54.0	53.8	47.4	42.3
9	162.1	165.0	164.7	168.5	183.7
10	41.3	43.9	42.2	44.6	40.7
11	121.0	124.2	123.7	124.9	126.3
12	203.3	199.1	196.9	188.4	204.3
13	52.5	48.5	49.3	131.7	42.0
14	139.6	64.9	65.4	151.6	55.7
15	139.5	138.1	139.1	140.0	139.9
16	114.3	115.8	116.5	114.4	115.8
17	23.8	23.3	19.6	15.4	13.2
18	114.0	115.7	113.0	115.3	113.9
19	22.3	23.2	24.8	23.7	25.7
20	24.6	27.2	21.2	24.7	20.0
3-OCH_3	51.6	52.1	51.7	52.0	52.1

[a] Measured at 100 MHz.
[b] Measured at 150 MHz.



Scheme 1. Proposed biogenetic pathway of compounds **1**–**7**.

Trigoflavidones B (**2**) and C (**3**) shared a molecular formula $C_{21}H_{28}O_4$ (with eight degrees of unsaturation, the same as in **1**) by analysis of their HRESIMS. By comparison of the spectral data of **2** and **3** with those of **1** (Tables 1 and 2) and a detailed analysis of 2D NMR spectra, it could be deduced that **2** and **3** were epoxides of **1**. The 1D NMR data of **2** and **3** were quite similar to those of **1**, except that the absence a trisubstituted double bond in **2** and **3**. The manifest changes, one oxygenated quaternary carbon (δ_C 54.0/53.8, C-8 in **2**/**3**) and an oxygenated methine [δ_H 3.16/3.18 (1H, s, H-14 in **2**/**3**); δ_C 64.9/65.4, C-14 in **2**/**3**], together with the same degrees of unsaturation as **1** demonstrated the appearance of the epoxide moiety in **2** and **3**. Key HMBC correlations of H-7 to C-14, H-11 to C-8 and C-13, H-14 to C-7, C-9, C-12, C-15 and C-17, H-15 to C-14, and H-17 to C-14 established the location of the epoxide moiety at C-8 and C-14.

The relative stereochemistry of **2** and **3** depicted in Fig. 3 were elucidated by the essential ROESY data. For **2**, the ROESY correlations of H_3-20/H-6α showed 1,3-diaxial *syn* relationships of the methyl group and H-6α, suggesting that the ring B took a chair conformation. The observed correlations of H-5/H_2-1 and H-5/H-6β indicated that H-5 was β-oriented. For **3**, the ring B also took a chair conformation with the β-configuration of the methyl propionate group and H-5 as deduced by the ROESY correlations of H_2-1/H-6β, H-5/H_2-1, H_3-20/H-18a, and H-7α/H-18a (Fig. 3). As no convincing evidence in the ROESY spectrum was available to assign the configuration of the epoxy group in **2** and **3**, a quantum chemical calculation of optical rotation (OR) values was applied[22–24] using the Gaussian03 program package.[25] The most-stable conformers of α- and β-epoxy isomers corresponding to **2** or **3** were calculated using the 'self-consistent reaction field' (SCRF) method in $CHCl_3$ solution at the B3LYP/6-311 G (2d,p) level. The calculated OR values for the α- and β-epoxy isomers were −70.2° and +49.5°, respectively, which are well comparable with those of experimentally recorded OR values for **2** (−68.9°) and 3 (+56.0°). Thus, the configuration of the epoxy group in **2** and **3** were α- and β-oriented, respectively.

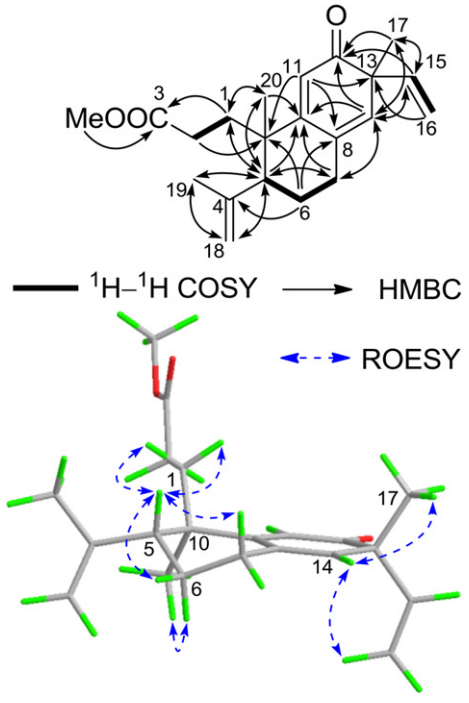


Fig. 2. Selected 2D NMR correlations for **1**.

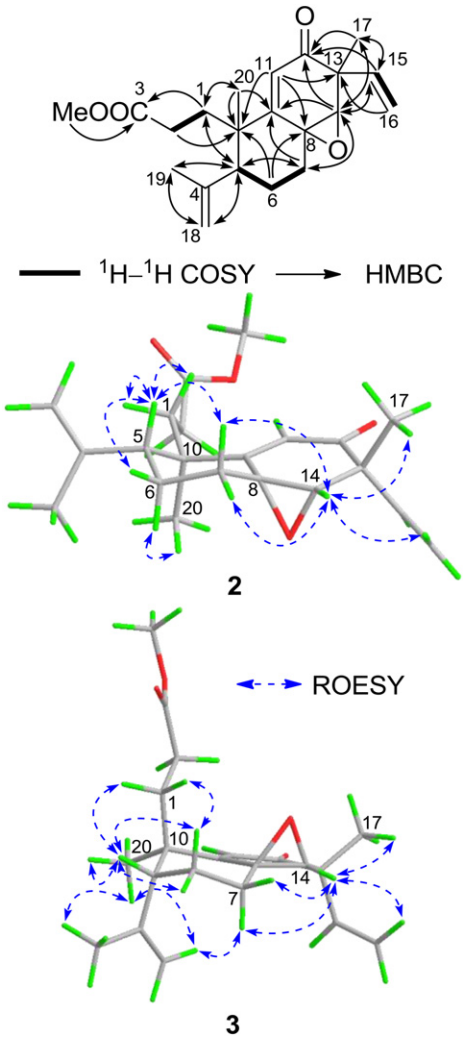


Fig. 3. Selected 2D NMR correlations for **2** and **3**.

The above assignment was supported by their opposite CD curves (see Supplementary data), which suggested that their CD properties were nearly exclusively contributed by the C-8 and C-14 chiral centers.

Positive HRESI-MS analysis of trigoflavidone D (**4**) indicated a molecular formula $C_{21}H_{28}O_3$, the same as **1**. Comparison of their 1D NMR data showed that the chemical shifts of 3,4-*seco*-ring A and ring B atoms did not change much (Tables 1 and 2). The appearance of an upfield chemical shift of the carbonyl (δ_C 188.4) together with its IR absorption band at 1664 cm^{-1} and the UV maximum of **4** (245 nm) indicated that the carbonyl was probably cross conjugated,[26] which was further confirmed by the HMBC correlations as shown in Fig. 4. The vinyl group, another common characteristic to **1** and **4**, was located at the quaternary carbon (C-8) by the HMBC correlations of H-7 and H-14 to C-15, H-15 to C-7, C-9, and C-14, H_2-16 to C-8. The location of the CH_3-17 group at C-13 was determined by the HMBC correlations of H-17 to C-12, C-13, and C-14, and H-14 to C-17. So compound **4** was determined to be a new 3,4-*seco* rearranged *ent*-pimarane type diterpenoid.

The stereochemistry of **4** was established as shown in Fig. 4 by the ROESY experiment. The relative configuration of the methyl group (CH_3-20) and H-5 in **4** was deduced as the same as in compounds **1**–**3** by the key ROESY correlations. The correlations of H_3-20/H-15 and H_3-20/H_2-16 implied that the vinyl group was α-orientation. The similar CD curves of **4** and trigonochinenes B and C[14] indicated the same absolute configurations for these compounds.

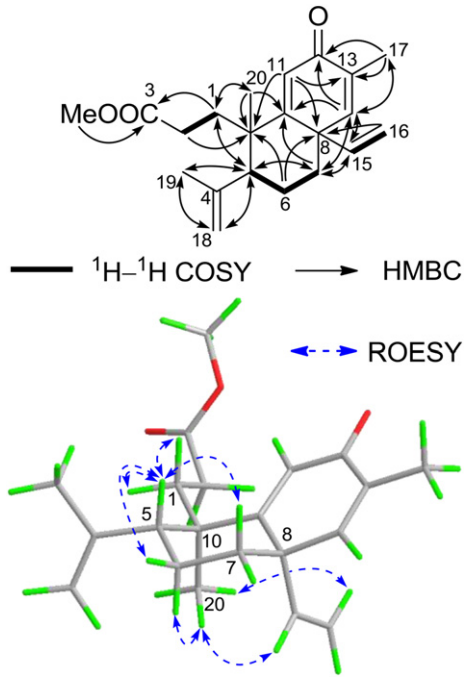


Fig. 4. Selected 2D NMR correlations for **4**.

Trigoflavidone E (**5**) had the molecular formula $C_{21}H_{28}O_3$ with eight degrees of unsaturation based on HREIMS analysis. Comparison of the 1D NMR spectra of compounds **5** and **1** revealed that a trisubstituted double bond in **1** was absent in **5** (Table 1), which suggested that **5** must have one additional ring. Analysis of the HMBC correlations (Fig. 2) involving H_2-7, H-11, H-14, the vinyl group, and the methyl groups (C-17 and C-20) constructed a five-membered ring (C_1 ring) with a conjugated carbonyl at C-12 fused by a cyclopropane ring (C_2 ring) substituted by the methyl (C-17) and the vinyl group at the quaternary carbon (C-14).

As shown in Fig. 5, the relative stereochemistry at the chiral centers of carbons C-5 and C-10 in **5** was determined to be the same as those of **1**–**4** by the cross-peaks observed in its ROESY spectrum. The correlations of H_3-17/H_2-1, H-13/H-15, and H-13/H-16b indicated that the methyl group was in a β-orientation and H-13 was α-oriented.

Due to the absence of applicable exciton coupling in the CD spectrum of **5** as well as no suitable model compounds found for reference, the absolute configuration of **5** cannot be resolved directly by the analysis of its CD curves. Thus, quantum chemical TDDFT was applied[5,27] to calculate the ECD spectrum of **5** and then compared with the experimental ECD spectrum (Fig. 6). The calculated and experimental CD curves match very well, which established the absolute configuration of **5**.

The rare 3,4-*seco*-diterpenoids (**1**–**7**) were isolated together with trigonoheterene[17] from *T. falvidus*, likely originated from the cleavage between C-3–C-4 bond of those of 3-keto precursors.[21,28] Referring to the confirmed Jacobs–Reynolds hypothesis,[26,29] and considering a close biogenetic relationships of the three skeletal types (i.e., the cleistanthane-, *ent*-pimarane-, and rearranged *ent*-pimarane-type diterpenoids), we proposed a plausible biosynthetic pathway for these 3,4-*seco*-diterpenoids in *T. flavidus* (see Scheme 1). Trigoflavidone A (**1**) could be modified through different biosynthetic pathways involving 1,2-shift or [1,3]-sigmatropic shift of the vinyl group and ring contraction rearrangement to form the other three skeletal types of 3,4-*seco*-diterpenoids **7** or **4** and **5**, respectively. Epoxidation of the 8,14 double bond would produce **2** and **3**. Additionally, the stereochemistry of the vinyl group in **1**–**3** could be deduced according to **4**, which yielded through the [1,3]-

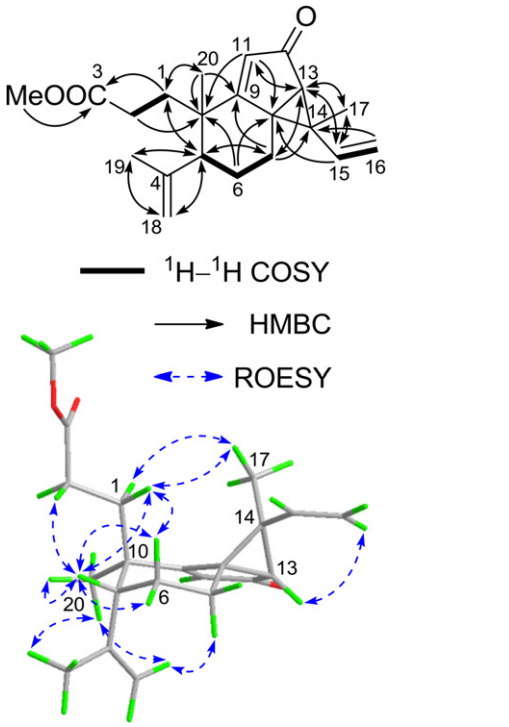


Fig. 5. Selected 2D NMR correlations for **5**.

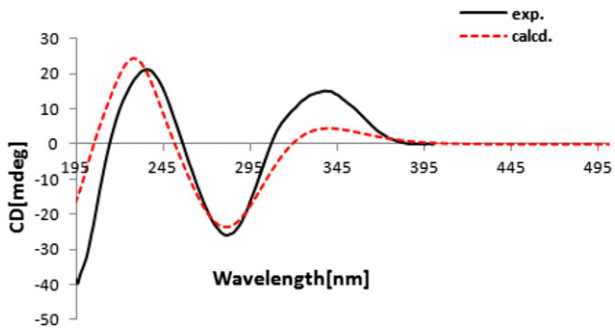


Fig. 6. The experimental ECD and calculated ECD spectra of **5**.

sigmatropic shift of the vinyl group from C-13 to C-8, suprafacially on the ring.

The cytotoxicities of **1**–**7** were preliminarily evaluated against five human tumor cell lines, HL-60 (premyelocytic leukemia), SMMC-7721 (hepatocellular carcinoma), A-549 (lung adenocarcinoma), MCF-7 (breast cancer), and SW480 (colon adenocarcinoma) by the MTT method[30] using *cis*-platin as positive control (with IC_{50} values of 1.14, 14.51, 12.76, 15.85, and 15.11 μM, respectively). Compound **7** showed weak inhibitory activities against the five tested human tumor cell lines with IC_{50} values of 15.48, 18.18, 22.77, 16.12, and 15.34 μM, respectively, while other compounds were inactive (IC_{50}>40 μM).

3. Experimental section

3.1. General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were detected on a Shimadzu UV 2401 spectrometer. CD spectra were recorded with an Applied Photophysics Chirascan spectrometer. IR spectra were determined on a Bruker Tensor-27 infrared spectrophotometer with KBr disks. 1D and 2D NMR spectra were recorded on Bruker AM-400, Bruker DRX-500, and Bruker Avance III 600 spectrometers using TMS as an internal standard. ESIMS and HRESIMS analyses were carried out on an API Qstar Pulsar 1 instrument. Semipreparative HPLC was performed on an Agilent 1200 series pump equipped with a diode array detector and a Zorbax SB-C_{18} column (5.0 μm, 9.4×250 mm). Silica gel (80–100 and 300–400 mesh, Qingdao Makall Group Co., Ltd.), MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical Corporation, Tokyo), C_{18} silica gel (40–75 μm, Fuji Silysia Chemical Ltd.), silica gel H (10–40 μm), and Sephadex LH-20 (GE Healthcare Bio-Xciences AB) were used for column chromatography. TLC spots were visualized under UV light and by dipping into 5% H_2SO_4 in EtOH followed by heating.

3.2. Computational details

All calculations were performed by the Gaussian03 program. For details, see Supplementary data.

3.3. Plant material

The stems of *T. flavidus* were collected from Sanya city, Hainan Province, the People's Republic of China, in October 2010. The plant was identified by one of the authors (G.-H.T.), and a voucher specimen (H20101011) was deposited at State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany.

3.4. Extraction and isolation

The air-dried and powdered stems of *T. flavidus* (16 kg) were extracted with MeOH for three times (4, 3, and 3 h, respectively) under reflux. The solution was combined and evaporated under reduced pressure to yield a residue, which was suspended in water and then partitioned successively with EtOAc and *n*-BuOH to give two corresponding portions. The EtOAc extract (110.0 g) was subjected to CC over silica gel (80–100 mesh) using petroleum ether/Me_2CO and $CHCl_3$/MeOH to yield six fractions (A–F). Fraction B was subjected to CC over MCI gel CHP 20P, Sephadex LH-20 and then further purified by repeated silica gel and semipreparative HPLC to obtain pure compounds **1** (52.5 mg), **2** (3.1 mg), **3** (9.2 mg), **4** (5.4 mg), **5** (2.9 mg), and **7** (750.5 mg). Compound **6** (940.4 mg) was obtained from fraction C by CC over C_{18} silica gel, Sephadex LH-20, and then repeated silica gel. The details on isolation of these compounds were provided in the Supplementary data.

*3.4.1. Trigoflavidone A (**1**).* Yellow oil; $[\alpha]_{22}^{D}$ +76.0 (*c* 0.95, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 325 (3.50), 240 (3.43) nm; CD (0.87 μM, MeOH), λ_{max} ($\Delta\varepsilon$) 202 (−55.36), 287 (−15.77), 341 (+3.75) nm; IR (KBr) ν_{max} 1739, 1660, 1642, 1437, 1381, 1299, 1261, 1197, 1175 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); ESIMS *m*/*z* 351 $[M+Na]^+$, HRESIMS *m*/*z* 351.1932 $[M+Na]^+$ (calcd for $C_{21}H_{28}O_3Na$, 351.1936).

*3.4.2. Trigoflavidone B (**2**).* Yellow oil; $[\alpha]_{22}^{D}$ −68.9 (*c* 0.21, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 255 (3.58), 232 (3.37), 192 (3.16) nm; CD (0.83 μM, MeOH), λ_{max} ($\Delta\varepsilon$) 200 (+20.81), 227 (+48.21), 275 (+3.15), 338 (−21.43) nm; IR (KBr) ν_{max} 1734, 1658, 1637, 1588, 1459, 1430, 1196, 1168 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); ESIMS *m*/*z* 367 $[M+Na]^+$, HRESIMS *m*/*z* 367.1880 $[M+Na]^+$ (calcd for $C_{21}H_{28}O_4Na$, 367.1885).

*3.4.3. Trigoflavidone C (**3**).* Yellow oil; $[\alpha]_{22}^{D}$ +56.0 (*c* 0.30, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 334 (2.40), 301 (2.54), 255 (3.83), 233 (3.58) nm; CD (0.21 μM, MeOH), λ_{max} ($\Delta\varepsilon$) 203 (−325.11), 230 (−33.80), 257 (+15.85), 341 (+36.39) nm; IR (KBr) ν_{max} 1739, 1670, 1628,

1450, 1438, 1198, 1175 cm^{-1}; ^1H and ^{13}C NMR data (see Tables 1 and 2); ESIMS *m/z* 367 $[M+Na]^+$, HRESIMS *m/z* 367.1879 $[M+Na]^+$ (calcd for $C_{21}H_{28}O_4Na$, 367.1885).

*3.4.4. Trigoflavidone D (**4**).* Yellow oil; $[\alpha]_{22}^{D}$ +147.7 (*c* 0.23, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 246 (3.99), 192 (3.42) nm; CD (0.51 μM, MeOH), λ_{max} ($\Delta\varepsilon$) 212 (+52.67), 226 (−38.31), 254 (+126.88), 344 (−5.89) nm; IR (KBr) ν_{max} 1738, 1664, 1638, 1451, 1437, 1377, 1197, 1173 cm^{-1}; ^1H and ^{13}C NMR data (see Tables 1 and 2); ESIMS *m/z* 351 $[M+Na]^+$, HRESIMS *m/z* 351.1934 $[M+Na]^+$ (calcd for $C_{21}H_{28}O_3Na$, 351.1936).

*3.4.5. Trigoflavidone E (**5**).* Yellow oil; $[\alpha]_{22}^{D}$ +120.0 (*c* 0.27, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 336 (2.65), 280 (3.62), 247 (3.64) nm; CD (0.91 μM, MeOH), λ_{max} ($\Delta\varepsilon$) 196 (−131.39), 236 (+70.06), 281 (−85.91), 337 (+49.86) nm; IR (KBr) ν_{max} 1739, 1692, 1657, 1649, 1641, 1452, 1440, 1197, 1173 cm^{-1}; ^1H and ^{13}C NMR data (see Tables 1 and 2); ESIMS *m/z* 351 $[M+Na]^+$, HRESIMS *m/z* 351.1932 $[M+Na]^+$ (calcd for $C_{21}H_{28}O_3Na$, 351.1936).

4. Cytotoxicity assays

Cytotoxicity evaluations were performed according to the previously described protocol.[31]

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Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.tet.2012.09.052.

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