JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Trigohowilols A−G, Degraded Diterpenoids from the Stems of *Trigonostemon howii*

Gui-Hua Tang,[†,‡] Yu Zhang,[†] Chun-Mao Yuan,[†] Yan Li,[†] Yu-Cheng Gu,[§] Ying-Tong Di,[†] Yue-Hu Wang,[⊥] Guo-Ying Zuo,[∥] Shi-Fei Li,[†,‡] Shun-Lin Li,[†] Hong-Ping He,*[,†] and Xiao-Jiang Hao*[,†]

[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, People's Republic of China

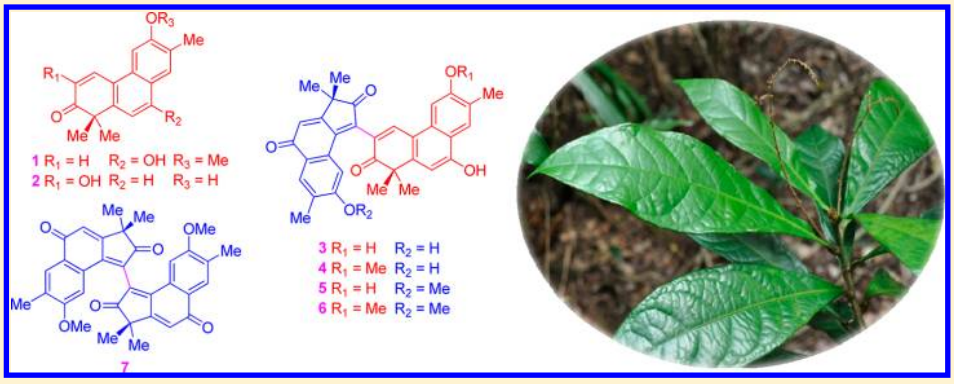
[‡]Graduate University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

[§]Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, U.K.

[⊥]Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, People's Republic of China

[∥]Research Center of Natural Medicine, Clinical School of Kunming General Hospital of Chengdu Military Command, Kunming, 650032, Yunnan, People's Republic of China

Supporting Information



ABSTRACT: Two new degraded diterpenoids, trigohowilols A (**1**) and B (**2**), four new heterodimers, trigohowilols C−F (**3**−**6**), one new homodimer, trigohowilol G (**7**), and three known degraded diterpenoids (**8**−**10**) were isolated from the methanol extract of the stems of *Trigonostemon howii*. Compounds **1**−**7** were evaluated for their cytotoxic activity against five human tumor cell lines by an MTT assay, and trigohowilols E (**5**) and F (**6**) exhibited inhibitory activity with IC_{50} values ranging from 2.33 to 12.57 μM. Moreover, compounds **1**−**6** showed weak antimicrobial activities (MIC values: 6.25−25 μg/mL) against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, MRSA 92#, and MRSA 98# using a 2-fold dilution method.

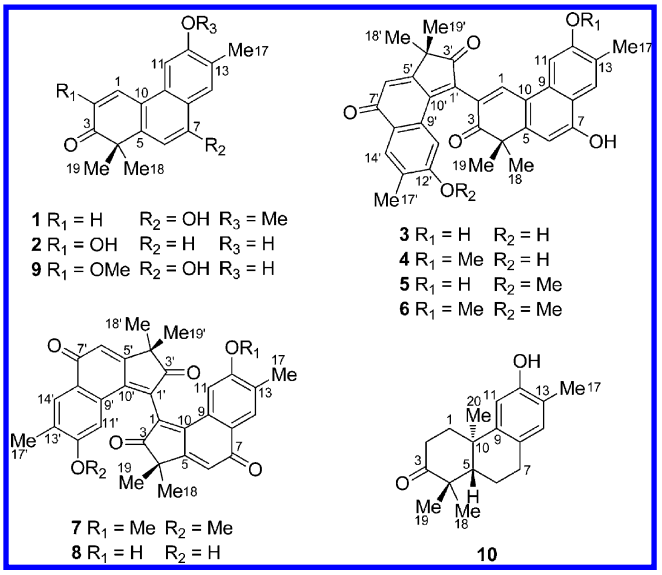
Trigonostemon howii Merrill & Chun (Euphorbiaceae), an evergreen shrub growing in dense montane forests, is distributed only in the Hainan Province in China.[1] Yue's group have recently reported 13 daphnane diterpenoids from this species.[2] Diterpenoids such as modified daphnane diterpenoids,[3−8] degraded diterpenoids,[9−11] and 3,4-*seco*-diterpenoids[9,12,13] as well as indole alkaloids[14−17] are the major classes of chemical constituents isolated from the genus *Trigonostemon*. These chemical components possess various bioactivities such as insecticidal,[3,18] antimicrobial,[9,11] cytotoxic,[5,19] and antiviral[6,8,20] properties, which have motivated natural product researchers to search for potential drug leads. In our continuing search for bioactive secondary metabolites from the plants of *Trigonostemon*,[7,10,11,14−17] the MeOH extract of *T. howii* was subjected to chromatographic procedures to yield seven new degraded diterpenoids including two monomers, trigohowilols A (**1**) and B (**2**), four heterodimers, trigohowilols C−F (**3**−**6**), a homodimer, trigohowilol G (**7**), and three known degraded diterpenoids, neoboutomannin (**8**),[21] 6,9-*O*-dedimethyltrigonostemone (**9**),[10] and 12-hydroxy-13-methylpodocarpa-9,11,13-trien-3-one (**10**).[22] This paper focuses on the isolation and structural elucidation of the seven new degraded diterpenoids, trigohowilols A−G (**1**−**7**), as well as their cytotoxic and antimicrobial activities.

RESULTS AND DISCUSSION

Trigohowilol A (**1**), a yellow powder, has the molecular formula $C_{18}H_{18}O_3$ with 10 indices of hydrogen deficiency based on the $[M]^+$ at *m*/*z* 282.1259 (calcd 282.1256) in its HREIMS. The IR spectrum indicated the presence of OH (3426 cm^{-1}), α,β-unsaturated carbonyl (1632 cm^{-1}), and aromatic (1589,

Received: September 12, 2012
Published: November 13, 2012

dx.doi.org/10.1021/np3006315 | J. Nat. Prod. 2012, 75, 1962−1966



1557, and 1464 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) displayed signals for four methyl groups including a *gem*-dimethyl group (δ_H 1.42, 6H), an aromatic methyl group (δ_H 2.32, 3H), and an *O*-methyl group (δ_H 4.04, 3H), three uncoupled aromatic protons (δ_H 7.02, 7.68, and 8.02, each 1H), and a pair of doublets (δ_H 6.02, 1H, d, J = 10.1 Hz and 8.47, 1H, d, J = 10.1 Hz). Eighteen signals consistent with four methyls, five methines, and nine quaternary carbons including a carbonyl, two oxygenated aromatic carbons, and a quaternary carbon of the *gem*-dimethyl group were observed in the ^{13}C NMR spectrum. Comparison of the NMR (Table 1) and MS data of **1** with those of **9**[10] demonstrated that **1** had the same skeleton as **9** except for the absence of the methoxy substituent at C-2. The $^1H-^1H$ COSY correlations of H-2 (δ_H 6.02) with H-1 (δ_H 8.47) and the HMBC correlations from H-1 to C-3, C-5, and C-9 and from H-2 to C-4 and C-10 (see Figure 1) further confirmed the above inference. In addition, the *O*-methyl group was located at C-12 by the HMBC correlation from 12-OCH_3 (δ_H 4.04, 3H, s) to C-12 (δ_C 159.4). Thus, the structure of **1** was established as shown in Figure 1.

Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR Data of 1 and 2 (δ in ppm)

	1[a]		**2**[b]	
position	δ_C, type	δ_H, multi (J in Hz)	δ_C, type	δ_H, multi (J in Hz)
1	140.8, CH	8.47, d (10.1)	114.8, CH	7.58, s
2	120.7, CH	6.02, d (10.1)	147.3, C	
3	204.1, C		201.8, C	
4	48.4, C		49.1, C	
5	150.1, C		142.2, C	
6	106.4, CH	7.02, s	121.9, CH	7.31, d (8.6)
7	156.3, C		128.2, CH	7.59, d (8.6)
8	119.2, C		123.2, C	
9	133.6, C		131.6, C	
10	115.6, C		129.0, C	
11	100.7, CH	7.68, s	104.5, CH	7.44, s
12	159.4, C		156.7, C	
13	127.6, C		128.5, C	
14	124.3, CH	8.02, s	130.6, CH	7.52, s
17	16.8, CH_3	2.35, s	16.6, CH_3	2.35, s
18/19	28.3, CH_3	1.42, s	28.4, CH_3	1.48, s
12-OCH_3	55.9, CH_3	4.04, s		

[a]Measured in acetone-d_6. [b]Measured in methanol-d_4.

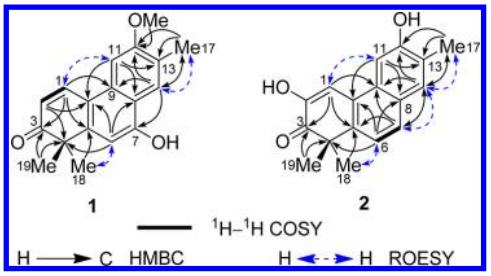


Figure 1. Selected 2D NMR correlations of **1** and **2**.

The molecular formula of compound **2** was determined as $C_{17}H_{16}O_3$ with 10 indices of hydrogen deficiency by analysis of the HREIMS data at $[M]^+$ m/z 268.1101 (calcd 268.1099). In the ^1H and ^{13}C NMR spectra of **2**, three methyls including a *gem*-dimethyl group (δ_H 1.48, 6H; δ_C 28.4, 2 × C), an aromatic methyl group (δ_H 2.35, 3H, δ_C 16.6), five methines, and nine quaternary carbons including a carbonyl (δ_C 201.8), an oxygenated olefinic carbon (δ_C 147.3), an oxygenated aromatic carbon (δ_C 156.7), and a quaternary carbon (δ_C 49.1) of the *gem*-dimethyl group were observed. The NMR spectroscopic features of compound **2** were analogous to those of **1** except for the appearance of two coupled aromatic protons (δ_H 7.31, 1H, d, J = 8.6 Hz and 7.59, 1H, d, J = 8.6 Hz) in **2** rather than two coupled olefinic protons in **1** and the missing *O*-methyl group in **2**. On the basis of the observed 2D NMR correlations (see Figure 1), the structure of **2** with the coupled aromatic protons at C-6 and C-7 as well as two OH groups at C-2 and C-11, respectively, was established and the compound trivially named trigohowilol B.

Trigohowilol C (**3**) was assigned the molecular formula $C_{33}H_{28}O_6$ with 20 indices of hydrogen deficiency by the $[M]^+$ ion at m/z 520.1888 (calcd 520.1886) by means of its HREIMS. Its IR spectrum showed absorption bands at ν_{max} 3432, 1688, 1635, 1625, 1585, 1576, and 1552 cm^{-1}, which accounted for the OH, carbonyl, and phenyl functionalities. In the 1D NMR spectra (Tables 2 and 3), there were signals for six methyls including two *gem*-dimethyl groups and two aromatic methyl groups, seven methines including two olefinic and five aromatic ones, 20 quaternary carbons including three carbonyls, four oxygenated aromatic carbons, and a pair of quaternary carbons of the *gem*-dimethyl groups, which were distinguished through analysis of the 2D NMR spectra. These data indicated that **3** was a heterodimer comprising two different highly aromatized degraded diterpenoids (**A** and **B**, see Figure 2). The structures of parts **A** and **B** were determined by the interpretation of HMBC and ROESY spectra (see Figure 2), which were similar to those of 6,9-*O*-dedimethyltrigonostemone (**9**)[10] and neoboutomannin (**8**),[21] respectively. The key HMBC correlation from H-1 to C-1′ confirmed that the two units were linked by the C-2–C-1′ bond to construct the heterodimer. Thus, the structure of **3** was established and named trigohowilol C.

The molecular formulas of trigohowilols D (**4**) and E (**5**) were determined as $C_{34}H_{30}O_6$ with 20 indices of hydrogen deficiency, by analyses of their HREIMS data. Comparison of the NMR (Tables 2 and 3) and MS data of **4** and **5** with those of **3** demonstrated that **4** and **5** had an additional *O*-methyl

dx.doi.org/10.1021/np3006315 | J. Nat. Prod. 2012, 75, 1962–1966

Table 2. ^{1}H NMR Data of 3−7 (δ in ppm)

	3[a]	4[a]	5[b]	6[c]	7[d]
position	δ_H, multi	δ_H, multi	δ_H, multi	δ_H, multi	δ_H, multi
1	8.41, s	8.75, s	8.59, s	8.83, s	
6	6.96, s	7.04, s	6.95, s	6.97, s	6.72, s
11	7.58, s	7.67, s	7.44, s	7.59, s	7.21, s
14	7.97, s	7.99, s	8.03, s	7.99, s	8.01, s
17	2.32, s	2.34, s	2.38, s	2.31, s	2.25, s
18	1.58, s	1.58, s	1.65, s	1.62, s	1.52, s
19	1.39, s	1.42, s	1.58, s	1.56, s	1.50, s
6′	6.59, s	6.62, s	6.55, s	6.46, s	6.71, s
11′	7.27, s	7.38, s	7.66, s	7.52, s	7.21, s
14′	7.88, s	7.89, s	8.01, s	7.87, s	8.01, s
17′	2.21, s	2.20, s	2.27, s	2.12, s	2.25, s
18′	1.38, s	1.38, s	1.44, s	1.40, s	1.52, s
19′	1.31, s	1.31, s	1.44, s	1.39, s	1.50, s
12-OMe		3.94, s		3.85, s	3.46, s
12′-OMe			3.42, s	3.34, s	3.46, s

[a]Measured in DMSO-d_6 at 600 MHz. [b]Measured in methanol-d_4 at 600 MHz. [c]Measured in methanol-d_4 at 500 MHz. [d]Measured in acetone-d_6 at 600 MHz.

group. The *O*-methyl group was located at C-12 in **4** and at C-12′ in **5** from analysis of their HMBC and ROESY correlations.

The HREIMS data of trigohowilol F (**6**) exhibited a molecular ion peak at *m/z* 548.2190 (calcd 548.2199), corresponding to the molecular formula $C_{35}H_{32}O_6$ with 20 indices of hydrogen deficiency. Comparing the NMR (Tables 2 and 3) and MS data of **6** and **3**, it appeared that the former had two additional *O*-methyl groups relative to **3**. The linkages of the two *O*-methyl groups to C-12 and C-12′ were determined by the HMBC correlations of the protons at δ_H 3.85 (3H, s, 12-OCH_3) to C-12 (δ_C 160.5) and the protons at δ_H 3.42 (3H, s, 12′-OCH_3) to C-12′ (δ_C 162.1), respectively, which were further confirmed by the ROESY spectrum.

The molecular formula of trigohowilol G (**7**), $C_{34}H_{30}O_6$ (with 20 indices of hydrogen deficiency), was determined by HREIMS (obsd $[M]^+$ *m/z* 534.2040). Similar to neoboutomannin (**8**),[21] its 1D NMR data showed only 17 carbon signals including four methyls, three methines, and 10 quaternary carbons, which indicated that **7** was also a symmetrical dimer. The difference between **7** and the known compound **8** was the presence of two *O*-methyl groups at C-12 and C-12′ in **7**. The structure of trigohowilol G was further established by the HSQC, HMBC, and ROESY spectra.

The cytotoxicities and antimicrobial activities of compounds **1**−**7** were evaluated in vitro. As shown in Table 4, compounds **5** and **6** showed cytotoxicities against human tumor cell lines with IC_{50} values in the range 2.33−12.57 μM, while compound **7** exhibited moderate activities (IC_{50} values: 2.78−17.75 μM). The structure−activity relationships of trigohowilols A−G (**1**−**7**) and neoboutomannin (**8**)[11] against the five human tumor cell lines indicated that the presence of an *O*-methyl group at C-12′ in the dimers (**5**−**7**), especially in the heterodimers (**5** and **6**), was important for their activities. Additionally, compounds **1**−**6** showed weak activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, MRSA (methicillin-resistant *Staph. aureus*) 92#, and MRSA 98# with MIC values ranging from 6.25 to 25 μg/mL.

EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra were detected on a Shimadzu UV 2401 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 analyses were determined on an API Qstar Pulsar 1 instrument. EIMS and HREIMS were carried out on a Waters Autospec Premier P776 mass spectrometer. Silica gel (80−100 and 300−400 mesh, Qingdao Makall Group Co., Ltd.), C_8 silica gel (20−45 μm, Fuji Silysia Chemical Ltd.), 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.

Plant Material. The stems of *T. howii* were collected in Sanya, Hainan Province, China, in October 2011. The plant was identified by one of the authors (G.-H.T.), and a voucher specimen (H20101201) was deposited at State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany.

Extraction and Isolation. The air-dried stems of *T. howii* (32 kg) were powdered and extracted with MeOH (3 × 35 L) under reflux three times (4, 3, and 3 h, respectively). The combined organic layers were evaporated under reduced pressure to give the crude MeOH extracts, which were suspended in H_2O and then partitioned with EtOAc and *n*-BuOH successively to give two corresponding portions (196.6 and 269.5 g). The EtOAc portion (196.6 g) was subjected to CC over silica gel (80−100 mesh) using petroleum ether−acetone (50:1 → 0:1) to afford five fractions (A−E).

Fractions B and D were subjected to CC over C_8 silica gel and Sephadex LH-20 and then further purified by repeated CC over silica gel to obtain pure compounds. Compounds **1** (14.0 mg), **2** (8.1 mg), **3** (7.5 mg), **4** (1.7 mg), **5** (3.0 mg), **6** (16.8 mg), **7** (3.8 mg), **9** (6.3 mg), and **10** (11.3 mg) were obtained from fraction B. Fraction D afforded compound **8** (7.0 mg).

*Trigohowilol A (**1**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 408 (3.99), 252 (4.52), 212 (4.52) nm; IR (KBr) ν_{max} 3426, 1632, 1589, 1557, 1491, 1464, 1429, 1383, 1354, 1253, 1233, 1213, 1161, 1056 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 305 $[M + Na]^+$; HREIMS *m/z* 282.1259 $[M]^+$ (calcd for $C_{18}H_{18}O_3$, 282.1256).

*Trigohowilol B (**2**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 400 (3.90), 279 (3.86), 244 (4.55), 215 (4.50), 196 (4.38) nm; IR (KBr) ν_{max} 3423, 1634, 1566, 1529, 1463, 1441, 1404, 1383, 1363, 1315, 1253, 1228, 1208, 1146, 1121, 1063 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 291 $[M + Na]^+$; HREIMS *m/z* 268.1101 $[M]^+$ (calcd for $C_{17}H_{16}O_3$, 268.1099).

*Trigohowilol C (**3**):* mauve, amorphous powder; UV (MeOH) λ_{max} (log ε) 502 (3.84), 433 (3.83), 283 (4.20), 255 (4.41), 213 (4.59) nm; IR (KBr) ν_{max} 3432, 1688, 1635, 1625, 1585, 1576, 1552, 1485, 1463, 1435, 1389, 1370, 1331, 1295, 1263, 1235, 1222, 1165, 1133, 1078 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 520 $[M]^+$ (27), 505 (6), 492 (9), 268 (14), 239, (23), 225 (17); HREIMS *m/z* 520.1888 $[M]^+$ (calcd for $C_{33}H_{28}O_6$, 520.1886).

*Trigohowilol D (**4**):* mauve, amorphous powder; UV (MeOH) λ_{max} (log ε) 478 (3.51), 426 (3.60), 283 (3.98), 254 (4.16), 212 (4.36) nm; IR (KBr) ν_{max} 3432, 1711, 1631, 1583, 1548, 1490, 1462, 1422, 1388, 1362, 1334, 1299, 1262, 1235, 1223, 1170, 1132, 1072 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 534 $[M]^+$ (11), 519 (1), 506 (2), 293 (27), 268 (6), 239, (4), 225 (9); HREIMS *m/z* 534.2037 $[M]^+$ (calcd for $C_{34}H_{30}O_6$, 534.2042).

*Trigohowilol E (**5**):* mauve, amorphous powder; UV (MeOH) λ_{max} (log ε) 503 (3.94), 433 (3.94), 283 (4.28), 256 (4.54), 212 (4.68) nm; IR (KBr) ν_{max} 3430, 1713, 1635, 1551, 1506, 1497, 1464, 1430, 1387, 1363, 1332, 1286, 1245, 1204, 1163, 1130, 1063 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 534 $[M]^+$ (100), 519 (11), 506 (23), 267 (6), 253, (6), 239 (8), 225 (10); HREIMS *m/z* 534.2036 $[M]^+$ (calcd for $C_{34}H_{30}O_6$, 534.2042).

*Trigohowilol F (**6**):* red-brown, amorphous powder; UV (MeOH) λ_{max} (log ε) 579 (4.06), 426 (4.07), 276 (4.42), 255 (4.61), 212 (4.78)

dx.doi.org/10.1021/np3006315 | J. Nat. Prod. 2012, 75, 1962−1966

Table 3. ^{13}C NMR Data of 3−7 (δ in ppm)

	3[a]	4[a]	5[b]	6[c]	7[d]
position	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type
1	142.3, CH	142.2, CH	144.8, CH	144.6, CH	135.6, C
2	120.5, C	121.5, C	122.5, C	123.0, C	
3	198.9, C	199.5, C	203.4, C	203.3, C	205.1, C
4	47.8, C	47.8, C	50.2, C	50.0, C	46.4, C
5	150.8, C	150.5, C	152.8, C	152.4, C	161.3, C
6	105.4, CH	106.0, CH	106.2, CH	106.7, CH	122.3, CH
7	157.7, C	157.7, C	158.8, C	159.2, C	183.5, C
8	117.7, C	118.2, C	119.7, C	119.9, C	126.1, C
9	133.0, C	133.0, C	135.0, C	134.8, C	130.7, C
10	112.7, C	113.9, C	114.3, C	115.0, C	151.4, C
11	103.5, CH	100.6, CH	104.0, CH	100.6, CH	108.4, CH
12	156.9, C	158.4, C	159.8, C	160.5, C	161.9, C
13	125.9, C	126.4, C	128.0, C	128.7, C	133.2, C
14	124.1, CH	123.7, CH	125.7, CH	125.1, CH	130.3, CH
17	16.6, CH_3	16.8, CH_3	17.0, CH_3	16.9, CH_3	16.6, CH_3
18	27.4, CH_3	27.0, CH_3	26.6, CH_3	26.5, CH_3	23.4, CH_3
19	29.9, CH_3	29.8, CH_3	31.8, CH_3	31.6, CH_3	24.8, CH_3
1′	137.0, C	138.6, C	141.6, C	141.5, C	135.6, C
3′	206.4, C	206.3, C	208.5, C	208.4, C	205.1, C
4′	44.5, C	44.4, C	46.2, C	46.1, C	46.4, C
5′	161.2, C	161.4, C	164.1, C	163.9, C	161.3, C
6′	119.4, CH	119.7, CH	120.9, CH	120.8, CH	122.3, CH
7′	183.1, C	183.1, C	185.8, C	185.6, C	183.5, C
8′	123.7, C	123.7, C	126.7, C	126.5, C	126.1, C
9′	130.9, C	130.8, C	132.7, C	132.5, C	130.7, C
10′	148.3, C	148.3, C	149.9, C	150.2, C	151.4, C
11′	113.6, CH	113.3, CH	109.3, CH	109.3, CH	108.4, CH
12′	159.2, C	159.4, C	162.5, C	162.1, C	161.9, C
13′	128.6, C	128.8, C	132.7, C	132.3, C	133.2, C
14′	129.1, CH	129.2, CH	130.4, CH	130.2, CH	130.3, CH
17′	16.3, CH_3	16.3, CH_3	16.7, CH_3	16.5, CH_3	16.6, CH_3
18′	23.4, CH_3	23.2, CH_3	23.7, CH_3	23.5, CH_3	23.4, CH_3
19′	23.54, CH_3	23.9, CH_3	24.7, CH_3	24.6, CH_3	24.8, CH_3
12-OMe		55.9, CH_3		56.0, CH_3	55.8, CH_3
12′-OMe			56.0, CH_3	55.9, CH_3	55.8, CH_3

[a]Measured in DMSO-d_6 at 150 MHz. [b]Measured in methanol-d_4 at 150 MHz. [c]Measured in methanol-d_4 at 100 MHz. [d]Measured in acetone-d_6 at 150 MHz.

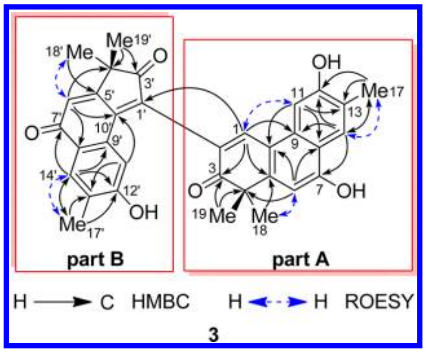


Figure 2. Selected 2D NMR correlations of 3.

nm; IR (KBr) ν_{max} 3429, 1714, 1645, 1597, 1554, 1488, 1463, 1421, 1385, 1363, 1334, 1235, 1166, 1130, 1065 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EISMS m/z 571 $[M + Na]^+$; HREIMS m/z 548.2190 $[M]^+$ (calcd for $C_{35}H_{32}O_6$, 548.2199).

Trigohowilol G (7): yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 365 (4.36), 325 (4.49), 276 (4.69), 211 (4.78) nm; IR (KBr) ν_{max} 1713, 1645, 1598, 1489, 1462, 1382, 1331, 1312, 1251, 1151, 1130, 1062 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; ESIMS m/z 557 $[M + Na]^+$; HREIMS m/z 534.2040 $[M]^+$ (calcd for $C_{34}H_{30}O_6$, 534.2042).

Cytotoxicity Assays. Compounds **1**−**7** were tested in vitro for their cytotoxicities against proliferation of 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), using the MTT assay.[23] Cytotoxicity evaluations were performed according to the previously described protocol.[24]

Antimicrobial Assays. The minimum inhibitory concentrations (MICs) of compounds **1**−**7** against *Staphylococcus aureus* (ATCC25923), *Pseudomonas aeruginosa* (ATCC27853) (National Institutes for Food and Drug Control (NIFDC), China), MRSA (methicillin-resistant *Staph. aureus*) 92#, and MRSA 98# (clinically isolated strains, from Kunming General Hospital of Chengdu Military Command) were determined by the 2-fold dilution method.[25] The strains used in antimicrobial tests were obtained from the Research Center of Natural Medicine, Clinical School of Kunming General Hospital of Chengdu Military Command. The protocols of antimicrobial tests were described previously.[11]

dx.doi.org/10.1021/np3006315 | J. Nat. Prod. 2012, 75, 1962−1966

Table 4. Antimicrobial Activities and Cytotoxicities of 1−7

	cytotoxicities (IC_{50} in μM)					antimicrobial activities (MIC in μg/mL)			
compound	HL-60	SMMC-7721	A-549	MCF-7	SW480	*Staph. aureus*	*P. aeruginosa*	MRSA 92#	MRSA 98#
1	24.84	>40	35.63	>40	>40	25	25	25	25
2	>40	>40	>40	>40	>40	25	25	50	50
3	>40	>40	>40	>40	>40	25	25	25	25
4	24.32	>40	>40	>40	>40	12.5	25	12.5	12.5
5	2.61	3.25	9.69	12.57	9.42	6.25	50	25	12.5
6	2.49	2.33	3.64	4.20	3.56	12.5	25	12.5	12.5
7	17.75	2.78	5.35	15.53	8.39	>50	>50	>50	>50
positive control	3.29[a]	9.62[a]	9.98[a]	15.92[a]	14.43[a]	0.78[b]	0.78[b]	0.78[b]	0.78[b]

[a] *cis*-Platin as positive control. [b] Vancomycin hydrochloride as positive control.

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR, MS, HREIMS, IR, and UV spectra of **1**−**7**; 1D NMR data of the known compounds **8** and **9**; a flowchart for the isolation of chemical constituents from *T. howii*. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel/Fax: +86-871-5223070. E-mail: haoxj@mail.kib.ac.cn; hehongping@mail.kib.ac.cn.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The Syngenta postgraduate studentship awarded to G.-H.T. (2010−2013, Kunming Institute of Botany, Chinese Academy of Sciences, Syngenta 3) is appreciated. This work was financially supported by the National Natural Science Foundation of China (30830114, 21072199), the National Basic Research Program of China (973 Program, 2009CB522300), the Natural Science Funding of Yunnan Province (2009CD112), the Foundation of Chinese Academy of Sciences to H.-P.H, and the Young Academic and Technical Leader Raising Foundation of Yunnan Province (2010CI047).

■ REFERENCES

(1) Li, B. T.; Gilbert, M. G. *Flora of China*; Science Press: Beijing, 2008; *Vol. 11*, pp 272−274.
(2) Dong, S. H.; Zhang, C. R.; Xu, C. H.; Ding, J.; Yue, J. M. *J. Nat. Prod.* **2011**, *74*, 1255−1261.
(3) Jayasuriya, H.; Zink, D. L.; Singh, S. B.; Borris, R. P.; Nanakorn, W.; Beck, H. T.; Balick, M. J.; Goetz, M. A.; Slayton, L.; Gregory, L.; Zakson-Aiken, M.; Shoop, W.; Singh, S. B. *J. Am. Chem. Soc.* **2000**, *122*, 4998−4999.
(4) Chen, H. D.; Yang, S. P.; He, X. F.; Ai, J.; Liu, Z. K.; Liu, H. B.; Geng, M. Y.; Yue, J. M. *Org. Lett.* **2010**, *12*, 1168−1171.
(5) Chen, H. D.; Yang, S. P.; He, X. F.; Liu, H. B.; Ding, J.; Yue, J. M. *Tetrahedron* **2010**, *66*, 5065−5070.
(6) Zhang, L.; Luo, R. H.; Wang, F.; Jiang, M. Y.; Dong, Z. J.; Yang, L. M.; Zheng, Y. T.; Liu, J. K. *Org. Lett.* **2010**, *12*, 152−155.
(7) Li, S. F.; Di, Y. T.; Li, S. L.; Zhang, Y.; Yang, F. M.; Sun, Q. Y.; Simo, J. M.; He, H. P.; Hao, X. J. *J. Nat. Prod.* **2011**, *74*, 464−469.
(8) Allard, P. M.; Martin, M. T.; Dau, M. E. T. H.; Leyssen, P.; Gueritte, F.; Litaudon, M. *Org. Lett.* **2012**, *14*, 342−345.
(9) Yin, S.; Su, Z. S.; Zhou, Z. W.; Dong, L.; Yue, J. M. *J. Nat. Prod.* **2008**, *71*, 1414−1417.
(10) Hu, X. J.; Wang, Y. H.; Kong, L. Y.; He, H. P.; Gao, S.; Liu, H. Y.; Ding, J.; Xie, H.; Di, Y. T.; Hao, X. J. *Tetrahedron Lett.* **2009**, *50*, 2917−2919.
(11) Tang, G. H.; Zhang, Y.; Gu, Y. C.; Li, S. F.; Di, Y. T.; Wang, Y. H.; Yang, C. X.; Zuo, G. Y.; Li, S. L.; He, H. P.; Hao, X. J. *J. Nat. Prod.* **2012**, *75*, 996−1000.
(12) Dong, S. H.; Liu, H. B.; Xu, C. H.; Ding, J.; Yue, J. M. *J. Nat. Prod.* **2011**, *74*, 2576−2581.
(13) Li, Y. X.; Mei, W. L.; Zuo, W. J.; Zhao, Y. X.; Dong, W. H.; Dai, H. F. *Phytochem. Lett.* **2012**, *5*, 41−44.
(14) Hu, X. J.; Di, Y. T.; Wang, Y. H.; Kong, L. Y.; Gao, S.; Li, C. S.; Liu, H. Y.; He, H. P.; Ding, J.; Xie, H.; Hao, X. J. *Planta Med.* **2009**, *75*, 1157−1161.
(15) Tan, C. J.; Di, Y. T.; Wang, Y. H.; Zhang, Y.; Si, Y. K.; Zhang, Q.; Gao, S.; Hu, X. J.; Fang, X.; Li, S. F.; Hao, X. J. *Org. Lett.* **2010**, *12*, 2370−2373.
(16) Li, S. F.; Di, Y. T.; He, H. P.; Zhang, Y.; Wang, Y. H.; Yin, J. L.; Tan, C. J.; Li, S. L.; Hao, X. J. *Tetrahedron Lett.* **2011**, *52*, 3186−3188.
(17) Li, S. F.; Cheng, Y. Y.; Zhang, Y.; Li, S. L.; He, H. P.; Hao, X. J. *Nat. Prod. Bioprospect.* **2012**, *2*, 126−129.
(18) Jayasuriya, H.; Zink, D. L.; Borris, R. P.; Nanakorn, W.; Beck, H. T.; Balick, M. J.; Goetz, M. A.; Gregory, L.; Shoop, W. L.; Singh, S. B. *J. Nat. Prod.* **2004**, *67*, 228−231.
(19) Lin, B. D.; Han, M. L.; Ji, Y. C.; Chen, H. D.; Yang, S. P.; Zhang, S.; Geng, M. Y.; Yue, J. M. *J. Nat. Prod.* **2010**, *73*, 1301−1305.
(20) Zhang, L.; Luo, R. H.; Wang, F.; Dong, Z. J.; Yang, L. M.; Zheng, Y. T.; Liu, J. K. *Phytochemistry* **2010**, *71*, 1879−1883.
(21) Tene, M.; Tane, P.; Tamokou, J. D.; Kuiate, J. R.; Connolly, J. D. *Phytochem. Lett.* **2008**, *1*, 120−124.
(22) Itokawa, H.; Ichihara, Y.; Takeya, K.; Morita, H.; Motidome, M. *Phytochemistry* **1991**, *30*, 4071−4073.
(23) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.
(24) Guo, L. L.; He, H. P.; Di, Y. T.; Li, S. F.; Cheng, Y. Y.; Yang, W.; Li, Y.; Yu, J. P.; Zhang, Y.; Hao, X. J. *Phytochemistry* **2012**, *74*, 140−145.
(25) Xu, S. Y.; Bian, R. L.; Chen, X. *Pharmacological Experiment Methodology*, 3rd ed.; People's Medical Publishing House: Beijing, 2002; pp 1647−1719.