Diterpenoids from the Feces of *Trogopterus xanthipes*

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Three new isopimarane diterpenoids, trogopteroids A−C (**1**−**3**), four new aromatic diterpenoids, trogopteroids D−G (**4**−**7**), and 12 known diterpenoids were isolated from the feces of *Trogopterus xanthipes.* Their structures were identified using spectroscopic methods. The relative configuration of **1** was confirmed by quantum calculations. Compound **1** represents the first example of a norisopimarane diterpenoid with an 8α,15α-olide ring. With the exception of compound **14**, all diterpenoids were evaluated for cytotoxicity against seven human tumor cell lines.

Human urine has been used for medical purposes in several countries for centuries, and small molecules, such as antineoplastones, have been isolated and evaluated in preclinical trials.[1] However, the potential medicinal properties of compounds present in the feces of other animals are relatively unknown. Wu-Ling-Zhi is the feces of the endangered *Trogopterus xanthipes* Milne-Edwards (Petauristidae). It is a basic constituent of 10 prescriptions (including three Tibetan ones) listed in the Pharmacopoeia of the People's Republic of China (2005) and has a long history of use in traditional Chinese medicine for the treatment of blood stasis and pain.[2] Previous reports indicated that the crude extract of Wu-Ling-Zhi possesses antitumor activity.[3] One diterpenoid and several triterpenoids have been isolated from an extract of the feces,[4−6] but the specific compounds responsible for the observed antitumor activity have yet to be identified. This paper describes the isolation, structure elucidation, and antitumor activity of 19 diterpenoids, including seven new analogues (**1**−**7**).

Results and Discussion

Trogopteroid A (**1**) was obtained as a white gum. Its formula was determined to be $C_{18}H_{22}O_3$ by HRESIMS, which indicated eight degrees of unsaturation. An IR absorption band at 1661 cm^{-1} suggested the presence of an α,β-unsaturated ketone moiety. The ^{13}C NMR and DEPT spectra showed 18 carbon signals attributed to three methyl, five methylene, three methine, and seven quaternary carbons. Comparison of the NMR spectrum of **1** with that of 8β-hydroxy-3-oxopimara-15-ene (**8**)[7] revealed that compound **1** is a dinorisopimarane diterpenoid. The spectra indicated that the carbon signals corresponding to a methyl, two sp^3 methylenes, an sp^3 methine, and a Δ^{15} bond in **8** were not present in **1**. Instead, two sp^2 quaternary carbons, two sp^2 methines, and an ester carbonyl group (δ_C 180.4) were observed. The COSY spectrum showed spin systems corresponding to H-1/H-2, H-6/H-7, and H-9/H-11/H-12. The HMBC correlations (Figure 1) between H_3-18/C-3 (δ_C 198.3), C-4 (δ_C 131.2), and C-5 (δ_C 152.8) and between H-7/C-5, C-6 (δ_C 128.2), C-8 (δ_C 83.2), C-9 (δ_C 44.9), and C-14 (δ_C 43.2) established the positions of the carbonyl group and two double bonds. Moreover, an ester carbonyl group was assigned to C-15 by its diagnostic chemical shift and the HMBC correlations observed between H_3-17/C-12, C-13, C-14, and C-15. In addition to three rings, two double bonds, one carbonyl, and one ester carbonyl group, the remaining single degree of unsaturation required that compound **1** had a lactone ring in the form of an 8,15-olide moiety, in agreement with the presence of a carbonyl absorption (1775 cm^{-1}) in the IR spectrum and a significant downfield signal for C-8 at δ_C 83.2. A molecular model study that accounted for ring strain suggested the presence of a *cis*-fused γ-lactone ring. However, the observed NOESY correlations between H_3-20/H-11/H-12/H_3-17 could not differentiate whether the molecule had an 8α,15α-olide or an 8β,15β-olide, which made the stereochemical assignment of the lactone ring more challenging. A key NOESY correlation between H_3-20/H-14a together with a weak correlation between H-7/H-14b, which is only possible for an 8α,15α-olide, defined the relative configurations at C-8 and C-13 (Figure 2). This conclusion was corroborated by quantum calculations using density functional theory (DFT) methods. The geometry was optimized at the B3LYP/6-31G(d) level.[8] The optimized structure was then used for ^{13}C NMR computations at the B3LYP/6-311+G(2d,p) level.[9] The calculated ^{13}C NMR data were corrected using a method described previously.[9] Relative errors in the chemical shifts were calculated between the corrected ^{13}C NMR data and the experimental results. The errors are summarized in the Supporting Information. The relative errors for **1b** (8β,15β-olide form) were generally larger than those for **1a** (8α,15α-olide form) (Supporting Information), and together with recent results,[10] structure **1a** was considered more reasonable. Importantly, the computed spatial distance between Me-20 and H-14 was 2.4 Å for **1a** and 4.7 Å for **1b** (Supporting Information), in good agreement with the observed NOESY correlation between H-20 and H-14a. Collectively, the evidence suggests that the structure of **1** is 3-oxo-16,19-norisopimara-4,6-dien-8α,15α-olide, which represents the first example of a norisopimarane diterpenoid with an 8α,15α-olide moiety.

Trogopteroids B (**2**) and C (**3**) were both isolated as white powders, and the formulas were determined to be $C_{20}H_{30}O_3$ and $C_{20}H_{28}O_3$, respectively, on the basis of their positive ionization HRESIMS. The ^{13}C NMR spectrum of **2** resembled that of **8**, and the spectra differed only in the oxygenation pattern of C-12. The carbonyl group was placed at C-12 in **2** and was absent in **8**, as determined by the HMBC correlations between H-11, H-15, and H-17 and C-12 (δ_C 214.1), which resulted in downfield shifts in C-11 and C-13. NMR data collected using DMSO-d_6 afforded a relatively sharp signal for the OH proton (δ 4.27, 1H, s), and additional relevant NOESY correlations between H-7a/OH/H-14a/H-17/H-11a/H-20 permitted conclusive assignment of the relative configuration at C-8 (Supporting Information). The above data

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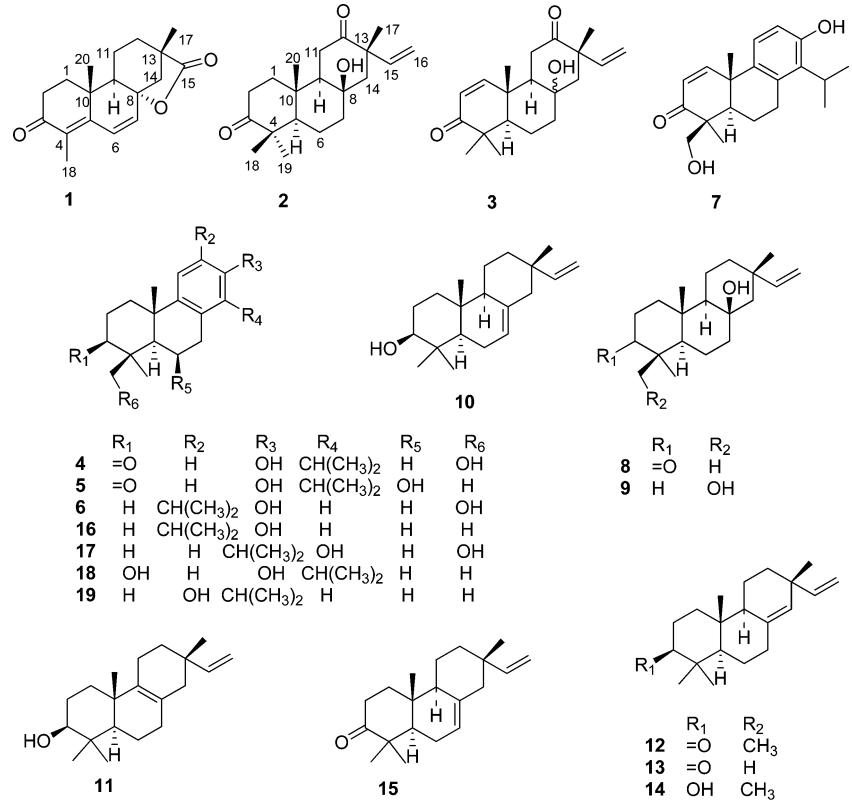
Chart 1



allowed **2** to be assigned the structure 8β-hydroxy-15-isopimaraene-3,12-dione. Compared with compound **2**, one more double bond was observed in **3**, consistent with the molecular formula, $C_{20}H_{28}O_3$, inferred from the positive HRESIMS. The double bond of **3** was placed at C-1, consistent with HMBC correlations between two vinylic protons (δ_H 6.95, d, $J = 10.4$ Hz; δ_H 5.87, d, $J = 10.4$ Hz) and C-3 (δ_C 204.5), which resulted in an upfield shift of C-3 ($\Delta\delta$ 12.0 ppm) attributed to the presence of a conjugated α,β-unsaturated carbonyl group. Due to the insufficient amount of compound **3**, the relative configuration at C-8 remained undetermined Accordingly, the structure of **3** was determined to be 8ζ-hydroxy-1,15-isopimaradiene-3,12-dione.

The molecular formula of trogopteroid D (**4**) was inferred to be $C_{20}H_{28}O_3$ on the basis of its positive HRESIMS. The IR spectrum

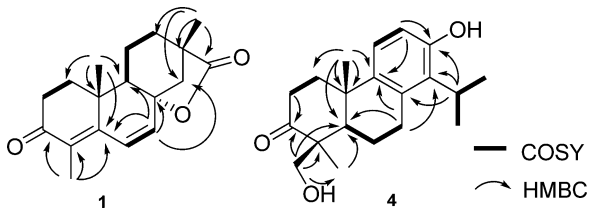


Figure 1. Important COSY and HMBC correlations in compounds **1** and **4**.

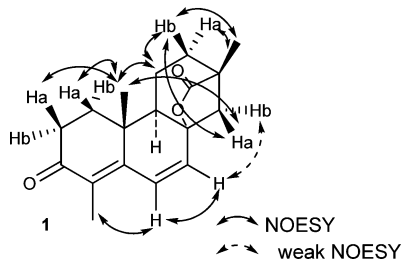


Figure 2. Important NOESY correlations in compound **1**.

of **4** showed the presence of hydroxy (3346 cm^{-1}) and carbonyl groups (1689 cm^{-1}) and a benzene ring (1601, 1585 cm^{-1}). The ^{13}C NMR and DEPT spectra showed 20 carbons attributed to four methyl, five sp^3 methylene, four methine (including two sp^3 and two sp^2), and six quaternary carbons (including two sp^3 and four sp^2) and one carbonyl group. These data are similar to those of 8,11,13-totaratriene-3,13-diol (**18**),[11] suggesting that **4** was a totarane-type diterpenoid, an oxidized form of **18**. The substituents at C-3 and C-18 were found to be a carbonyl and a hydroxy group in **4**, respectively, corresponding to the observations of HMBC correlations between H-2, H$_2$-18 (δ_H 4.08, d, $J = 14.0$; δ_H 3.50, d, $J = 14.0$), and H$_3$-19/C-3 (δ_C 221.0) and the ROESY response of H$_2$-18/H$_3$-20. Therefore, the structure of **4** was determined to be 13,18-dihydroxy-8,11,13-totaratrien-3-one.

Trogopteroids **4** and **5** had the same molecular composition ($C_{20}H_{28}O_3$). Analysis of their NMR data revealed that they shared the same diterpenoid skeleton, differing only in the location of a hydroxy group. In compound **5**, the hydroxy group was present at C-6 instead of C-18 in compound **4**, as indicated by the HMBC correlations in **5** between H$_3$-18, H$_3$-19, and C-3, between H-5 and C-6 (δ_C 65.7) and C-7, and between H-7 and C-6, C-8, and C-9. The hydroxy group at C-6 was β-oriented, as indicated by the NOE responses of H-5α/H-19α/H-6. In addition, H-5 exhibited a slightly broadened singlet in the ^{1}H NMR spectrum of **5**, which might have arisen from the ca. 90° dihedral angle between H−C-5−H−C-6. Therefore, the structure of **5** was determined to be 6β,13-dihydroxy-8,11,13-totaratrien-3-one. In the same manner, diterpenoid **7** was determined to be an analogue of **4**, differing only in the presence of a double bond at C-1 in **7**, as indicated by the HMBC spectrum, which resulted in an upfield shift for C-3 ($\Delta\delta$ 14.9 ppm). Accordingly, the structure of **7** was assigned as 13,18-dihydroxy-1,8,11,13-totaratetraen-3-one.

The molecular formula of trogopteroid F (**6**) was established as $C_{20}H_{30}O_2$ from its HRESIMS. The ^{13}C NMR data of **6** were similar to those of sempervirol (**16**),[12] differing from **16** only in the replacement of the Me-18 group by a hydroxymethyl moiety. This

Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1**−**3** in $CDCl_3$

	1[a]		**2**[b]		**3**[b]	
	δ_H (*J* in Hz)	δ_C mult	δ_H (*J* in Hz)	δ_C mult	δ_H (*J* in Hz)	δ_C mult
1a	2.07, dt (10.6, 6.0)	33.4, t	1.87, overlap	37.6, t	6.95, d (10.4)	156.3, d
1b	1.72, dt (10.6, 6.0)		1.39, overlap			
2a	2.58, m	32.9, t	2.56, m	33.8, t	5.87, d (10.4)	126.1, d
2b	2.53, m		2.40, m			
3		198.3, s		216.5, s		204.5, s
4		131.2, s		47.3, s		44.6, s
5		152.8, s	1.39, overlap	54.5, d	1.43, t (4.8)	54.2, d
6a	6.69, d (10.0)	128.2, d	1.81, d (12.4)	18.7, t	1.84, m	18.1, t
6b			1.52, m		1.69, m	
7a	6.03, d (10.0)	135.0, d	1.87, overlap	41.8, t	1.85, overlap	41.8, t
7b			1.51, m		1.55, m	
8		83.2, s		71.4, s		72.1, s
9	2.11, t (7.0)	44.9, d	1.42, m	54.2, d	1.65, m	49.4, d
10		38.4, s		36.8, s		39.4, s
11a	1.92, overlap	17.7, t	2.96, t (14.0)	34.7, t	3.07, t (14.0)	34.4, t
11b			2.23, dd (14.0, 3.6)		2.40, dd (14.0, 3.6)	
12a	1.93, overlap	33.1, t		214.1, s		213.2, s
12b	1.62, m					
13		42.1, s		50.3, s		50.3, s
14a	2.30, d (12.0)	43.2, t	1.87, overlap	52.5, t	1.85, overlap	52.6, t
14b	2.01, d (12.0)		1.69, d (14.4)		1.71, d (14.4)	
15		180.5, s	6.08, dd (17.6, 10.9)	143.1, d	6.08, dd (17.6, 10.9)	142.9, d
16a			5.11, d (10.9)	112.8, t	5.12, d (10.9)	113.0, t
16b			5.00, d (17.6)		5.02, d (17.6)	
17	1.25, s	20.8, q	1.48, s	25.0, q	1.49, s	25.2, q
18	1.87, br s	10.8, q	1.09, s	21.5, q	1.15, s	21.6, q
19			1.11, s	26.6, q	1.16, s	27.9, q
20	1.21, br s	19.1, q	1.14, s	14.6, q	1.23, s	18.0, q

[a] ^{1}H at 500 MHz and ^{13}C at 100 MHz. [b] ^{1}H at 400 MHz and ^{13}C at 100 MHz.

Table 2. ^{1}H and ^{13}C NMR Data for Compounds **4**−**7** in $CDCl_3$

	4[a]		**5**[b]		**6**[b]		**7**[b]	
	δ_H (*J* in Hz)	δ_C mult.	δ_H (*J* in Hz)[a]	δ_C mult.	δ_H (*J* in Hz)	δ_C mult.	δ_H (*J* in Hz)	δ_C mult.
1a	2.42, m	38.0, t	2.22, m	40.6, t	2.32, d-like (12.8)	39.1, t	7.65, d (10.0)	160.4 d
1b	1.99, m		1.89, m		1.41, dt (12.8, 4.0)			
2a	2.70, m	35.0, t	2.63, d-like (8.0)	34.5, t	1.62, m	19.0, t	6.03, d (10.0)	126.5, d
2b	2.58, m		2.62, d-like (8.0)		1.45, m			
3a		221.0, s		217.7, s	1.87, d-like (13.5)	35.2, t		206.1, s
3b					1.01, dd (13.5, 4.0)			
4		50.6, s		47.7, s		38.6, s		48.5, s
5	2.09, d (16.0)	50.5, d	1.98, s	51.9, d	1.47, d (13.2)	51.5, d	2.24, dd (13.0, 1.6)	47.6, d
6a	1.91, m	19.8, t	4.56, s	65.7, d	1.94, dd (13.2, 6.8)	19.1, t	2.02, m	19.2, t
6b	1.64, m				1.67, m		1.78, m	
7a	3.00, dd (17.0, 6.5)	29.1, t	3.11, d (17.5)	39.3, t	2.83, m	30.5, t	3.02, dd (17.2, 6.4)	28.9, t
7b	2.71, m		3.01, dd (17.5, 5.0)		2.76, m		2.81, m	
8		131.0, s		128.9, s		142.3, s		132.1, s
9		139.5, s		138.4, s		133.4, s		135.6, s
10		31.2, s		31.2, s		37.4		31.2, s
11	6.97, d (10.5)	124.4, d	7.06, d (11.0)	124.7, d	7.07, s	122.6, d	7.15, d (8.4)	123.4, d
12	6.56, d (10.5)	115.0, d	6.63, d (11.0)	115.4, d		131.9, s	6.63, d (8.4)	114.8, d
13		152.4, s		152.9, s		150.3, s		152.9, s
14		133.5, s		131.7, s	6.41, s	114.8, d		134.3, s
15	3.25, m	27.4, d	3.24, m	27.6, d	3.13, m	27.2, d	3.27, m	27.4, d
16	1.34, d (6.8)	20.2, q	1.36, d (3.0)	20.2, q	1.22, d (1.5)	22.6, q	1.34, d (6.8)	20.1, q
17	1.35, d (6.8)	20.3, q	1.38, d (3.0)	20.4, q	1.24, d (1.5)	22.8, q	1.35, d (6.8)	20.2, q
18	4.08, d (14.0)	65.7, t	1.22, s	22.4, q	3.85, d (11.5)	65.3, t	4.04, d (11.2)	65.2, t
	3.50, d (14.0)				3.54, d (11.5)		3.58, d (11.2)	
19	1.35, s	20.3, q	1.42, s	28.6, q	1.04, s	26.8, q	1.38, s	22.3, q
20	1.26, s	25.9, q	1.52, s	25.9, q	1.17, s	25.9, q	1.40, s	30.4, q

[a] ^{1}H at 500 MHz and ^{13}C at 100 MHz. [b] ^{1}H at 400 MHz and ^{13}C at 100 MHz.

was confirmed by the HMBC couplings between H_2-18/C-3, C-4, C-5, and C-19 and the NOE response of H_3-19/H-5.

Known diterpenoids were identified as 8β-hydroxy-3-oxopimara-15-ene (**8**),[7] akhdardiol (**9**),[13] isopimara-7(8),15-dien-3β-ol (**10**),[14] isopimara-8,15-dien-3β-ol (**11**),[15] isopimara-8(14),15-dien-3-one (**12**),[16] 19-norisopimara-8(14),15-dien-3-one (**13**),[17] 3α-hydroxy-isopimara-8(14),15-dien-3-one (**14**),[18] isopimara-7,15-dien-3-one (**15**),[19] sempervirol (**16**),[12] macrophynin E (**17**),[20] 8,11,13-totaratriene-3,13-diol (**18**),[11] and ferruginol (**19**)[21] by comparison with literature data.

With the exception of compound **14**, all other diterpenoids were evaluated *in vitro* for their cytotoxicity against HL-60, K562, U937, HepG2, MCF-7, and SGC7901 human cancer cell lines, with 10-hydroxycamptothecin used as a positive control. The results of the cytotoxicity assays (Supporting Information) showed that only compounds **15**, **18**, and **19** exhibited moderate or weak cytotoxic

activity (IC_{50} 19.7−35.1 μM) toward some cancer cell lines, whereas most of the compounds showed no significant activity (IC_{50} > 40 μM).

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu double-beam 210A spectrometer. IR spectra were measured on a Tensor 27 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker AV-400 or a DRX-500 spectrometer. EIMS were determined on a Finnigan-4510 spectrometer. ESIMS and HRESIMS were recorded with an API QSTAR Pulsar 1 spectrometer. Silica gel (200−300 mesh, Qingdao Marine Chemical Inc., People's Republic of China), RP-18 (40−60 μm, Daiso Co., Japan), and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Semipreparative HPLC was carried out on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18} column (9.4 mm ×25 cm, i.d.).

Material. The diet of *T. xanthipes* was the leaves of pine or cypress trees. The feces of *T. xanthipes* were collected from a national breeding base of *T. xanthipes* in Shangzhou County, Shanxi Province, People's Republic of China, in September 2007 and authenticated by one of the authors (X.-J.Z.). A voucher specimen (CHYX0233) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation. The air-dried powders of the feces (20 kg) were extracted with 80% acetone (3 × 35 L, each 10 d) at room temperature. The combined extracts were concentrated to afford a crude extract (950 g), which was partitioned in EtOAc/H_2O (1:1) to obtain an EtOAc extract (330 g). The EtOAc extract was subjected to column chromatography (CC) on silica gel using increasing amounts (2%) of MeOH in $CHCl_3$ and finally MeOH as the eluent to produce fractions A−F. Fraction A (2.8 g) was separated into fractions A1−A3 by CC over silica gel with petroleum ether/EtOAc (50:1−25:1) as the eluent. Fraction A1 (4.7 g) was gel filtered over Sephadex LH-20 ($CHCl_3$/MeOH, 6:4), followed by semipreparative RP-HPLC (MeOH/H_2O, 90%), to give compounds **4** (28.6 mg) and **7** (13.2 mg). Fraction A2 (1.5 g) was separated by CC over silica gel with petroleum ether/EtOAc (70:1−30:1) as the mobile phase to yield subfractions A21 (220 mg) and A22 (340 mg). Compound **15** (25 mg) was purified from fraction A21 by preparative TLC using petroleum ether/^{i}PrOH (15:1) as the development solvent. Fraction A22 was further subjected to silica gel CC and eluted with petroleum ether/^{i}PrOH (45:1−25:1) to give **14** (1.2 mg), **16** (12.6 mg), and a fraction containing **17**. Pure compound **17** (13.2 mg) was obtained by further semipreparative HPLC (MeOH/H_2O, 85%). Fraction B (4.2 g) was divided into subfractions B1 and B2 (121 and 156 mg, respectively) by silica gel CC using petroleum ether/EtOAc (70:1−25:1) as the eluent. Compound **5** (8.7 mg) was purified from fraction B1 by RP-18 column using aqueous MeOH as the mobile phase (75%−90%). Fraction B2 was fractioned by passage over a column containing silica gel (petroleum ether/EtOAc, 30:1−15:1 eluent) followed by a semipreparative HPLC (MeOH/H_2O, 85%) to give pure **6** (38.0 mg), **10** (33.4 mg), and **11** (4.2 mg). Fraction C (2.2 g) was initially separated by CC over silica gel, eluted with petroleum ether/^{i}PrOH (40:1−15:1), and further purified via an RP-18 column (MeOH/H_2O, 72%−85%) to give **8** (40.3 mg) and **9** (7.8 mg). Fraction D (1.3 g) was divided into two subfractions, D1 and D2 (40 and 132 mg, respectively), by silica gel CC with a gradient of petroleum ether/EtOAc (20:1−5:1) as eluent. Compound **1** (6.1 mg) was obtained from fraction D1 by an RP-18 column (MeOH/H_2O, 60%−80%). In the same manner, a mixture containing **2** and **3** was isolated from fraction D2, which was further purified by RP-HPLC (AcCN/H_2O, 60%) to produce **2** (6 mg) and **3** (2 mg). Fraction E (1.3 g) was separated by silica gel CC and eluted with petroleum ether/Me_2CO (35:1−15:1) to provide subfractions E1 (56 mg) and E2 (43 mg). Compounds **13** (3 mg) and **18** (19.3 mg) were purified from fractions E1 and E2, respectively, by RP-18 column using aqueous MeOH (60%−80%) as the eluent. Separation of fraction F (980 mg) by silica gel CC (petroleum ether/EtOAc, 18:1−5:1), in combination with final semipreparative HPLC (MeOH/H_2O, 75%), yielded compounds **12** (4.1 mg) and **19** (2.2 mg).

Trogopteroid A (1): white gum; $[\alpha]_D^{24}$ +179.2 (*c* 0.39, MeOH); UV (MeOH) λ_{max} (log ε) 283 (4.16) nm; IR (KBr) ν_{max} 2963, 2930, 2871, 1775, 1661, 1451, 1208, 1151, 909 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m/z* 286 $[M]^+$ (77), 242 $[M - CO_2]^+$ (100), 227 $[M - CO_2 - CH_3]^+$ (96), 209 (45), 199 (46), 185 (76), 171 (82), 157 (39), 143 (36); HRESIMS (positive) *m/z* 287.1647 $[M + H]^+$ (calcd for $C_{18}H_{23}O_3$, 287.1647).

Trogopteroid B (2): white powder; $[\alpha]_D^{24}$ +41.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 284 (2.58), 202 (3.45) nm; IR (KBr) ν_{max} 3438, 2975, 2949, 2917, 1706, 1686, 1640, 1456, 1386, 1122, 915 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS (positive) *m/z* 341 $[M + Na]^+$; HRESIMS (positive) *m/z* 341.2092 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_3Na$, 341.2092).

Trogopteroid C (3): white powder; $[\alpha]_D^{24}$ +27.7 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 226 (3.89) nm; IR (KBr) ν_{max} 3440, 2971, 2930, 2867, 1705, 1694, 1653, 1458, 1122, 921 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS (positive) *m/z* 339 $[M + Na]^+$ (**3**); HRESIMS (positive) *m/z* 339.1959 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_3Na$, 339.1936).

Trogopteroid D (4): white powder; $[\alpha]_D^{24}$ +17.4 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 285 (3.36), 279 (3.37) nm; IR (KBr) ν_{max} 3346, 2951, 2926, 2869, 1689, 1601, 1585, 1451, 1282, 1038, 806 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS (70 eV) *m/z* 316 $[M]^+$ (24), 301 $[M - CH_3]^+$ (25), 286 $[M - 2CH_3]^+$ (29), 271 $[M - 3CH_3]^+$ (100), 229 (38), 199 (23), 159 (33), 97 (32); HRESIMS (positive) *m/z* 339.1928 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_3Na$, 339.1936).

Trogopteroid E (5): white powder; $[\alpha]_D^{24}$ +50.0 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 283 (3.43) nm; IR (KBr) ν_{max} 3434, 2956, 2924, 2854, 1695, 1589, 1457, 1379, 1281, 815 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS (70 eV) *m/z* 316 $[M]^+$ (45), 301 $[M - CH_3]^+$ (25), 283 (21), 259 (21), 255 (100), 241 (37), 199 (55), 159 (21), 149 (20); HRESIMS (positive) *m/z* 339.1933 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_3Na$, 339.1936).

Trogopteroid F (6): white powder; $[\alpha]_D^{24}$ +105.6 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 281 (3.58) nm; IR (KBr) ν_{max} 3422, 2961, 2958, 2870, 2853, 1721, 1616, 1582, 1460, 1418, 1246, 1019 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS (70 eV) *m/z* 302 $[M]^+$ (37), 287 $[M - CH_3]^+$ (100), 269 $[M - CH_3 - H_2O]^+$ (33), 227 (25), 175 (46), 157 (16), 147 (20), 133 (15); HRESIMS (positive) *m/z* 303.2321 $[M + H]^+$ (calcd for $C_{20}H_{31}O_2$, 303.2324).

Trogopteroid G (7): white powder; $[\alpha]_D^{25}$ +105.6 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 284 (3.32), 223 (4.17) nm; IR (KBr) ν_{max} 3423, 2958, 2928, 2871, 1723, 1654, 1586,1451, 1377, 1284, 1187, 1076, 1040, 817 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS (70 eV) *m/z* 314 $[M]^+$ (30), 299 $[M - CH_3]^+$ (82), 269 (69), 241 (32), 227 (100), 209 (21), 199 (16), 165 (17); HRESIMS (positive) *m/z* 315.1959 $[M + H]^+$ (calcd for $C_{20}H_{27}O_3$, 315.1960).

Cytotoxicity Assay. The cytotoxicity assay was performed according to the modified MTT method.[22−24] Briefly, 100 μL of adherent cells was seeded into 96-well microtiter plates and allowed to adhere for 12 h before drug addition, whereas suspended cells were seeded immediately prior to drug addition, with an initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the tested compounds at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM for 48 h. After the treatment period, cell viability was detected and IC_{50} values were calculated as described in the literature.[25]

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Supporting Information Available: NOESY correlations in compound **2**. Cytotoxic activity of compounds **1**−**19** with the exception of **14**. An illustration of the quantum calculations of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Burzynski, S. R. *Int. J. Exp. Clin. Chemother.* **1989**, *2*, 63–69.
(2) Li, S. Z. *Compendium of Materia Medica*; People's Medical Publishing House: Beijing, 1982; p 2642.
(3) Kosuge, T.; Yokota, M.; Sugiyama, K.; Yamamoto, T.; Ni, M. Y.; Yan, S. C. *Yakugaku Zasshi* **1985**, *105*, 791–795.

(4) Yang, D. M.; Su, S. W.; Li, X.; Zhu, R. *Acta Pharm. Sin.* **1987**, *22*, 756–760.
(5) Numata, A.; Yang, P. M.; Takahashi, C.; Fujiki, R.; Nabae, M.; Fujita, E. *Chem. Pharm. Bull.* **1989**, *37*, 648–651.
(6) Numata, A.; Takahashi, C.; Miyamoto, T.; Yoneda, M.; Yang, P. M. *Chem. Pharm. Bull.* **1990**, *38*, 942–944.
(7) Yang, H. O.; Sun, D. Y.; Han, B. H. *Planta Med.* **1995**, *61*, 37−40.
(8) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; et al. Gaussian 03 User's Reference; Gaussian Inc.: Carnegie, PA, 2003.
(9) (a) Timmons, C.; Wipf, P. *J. Org. Chem.* **2008**, *73*, 9168–9170. (b) Chen, L. X.; Zhu, H. J.; Wang, R.; Zhou, K. L.; Jing, Y. K.; Qiu, F. *J. Nat. Prod.* **2008**, *71*, 852–855. (c) Maulucci, N.; Izzo, I.; Bifulco, G.; Aliberti, A.; De Cola, C.; Comegna, D.; Gaeta, C.; Napolitano, A.; Pizza, C.; Tedesco, C.; Flot, D.; De Riccardis, F. *Chem. Commun.* **2008**, *33*, 3927–3929. (d) Pu, J. X.; Huang, S. X.; Ren, J.; Xiao, W. L.; Li, L. M.; Li, R. T.; Li, L. B.; Liao, T. G.; Lou, L. G.; Zhu, H. J.; Sun, H. D. *J. Nat. Prod.* **2007**, *70*, 1706–1711. (e) Hua, Y.; Ren, J.; Chen, C. X.; Zhu, H. J. *Chem. Res. Chin. Univ.* **2007**, *23*, 592–596.
(10) Sheldrake, H. M.; Jamieson, C.; Burton, J. W. *Angew. Chem., Int. Ed.* **2006**, *45*, 7199–7202.
(11) Liu, R.; Wang, C. Z.; Ouyang, M. A. *Nat. Prod. Res. Dev.* **2006**, *18*, 187–195.
(12) Lin, Y. T.; Kuo, Y. H.; Chang, B. H. *J. Chin. Chem. Soc.* **1976**, *22*, 331–334.
(13) Stierle, D. B.; Stierle, A. A.; Larsen, R. D. *Phytochemistry* **1988**, *27*, 517–522.
(14) Liu, R. M. *Acta Bot. Yunnan* **1984**, *6*, 219–222.
(15) Asili, J.; Lambert, M.; Ziegler, H. L.; Staerk, D.; Sairafianpour, M.; Witt, M.; Asghari, G.; Ibrahimi, I. S.; Jaroszewski, J. W. *J. Nat. Prod.* **2004**, *67*, 631–637.
(16) Kono, Y.; Takeuchi, S.; Kodama, O.; Sekido, H.; Akatsuka, T. *Agric. Biol. Chem.* **1985**, *49*, 1695–1701.
(17) Torbjorn, N.; Bjorn, W. *Acta Chem. Scand.* **1971**, *5*, 611–614.
(18) Ansell, S. M.; Pegel, K. H.; Taylor, D. A. H. *Phytochemistry* **1993**, *32*, 953–959.
(19) Lago, J. H. G.; Brochini, C. B.; Roque, N. F. *Phytochemistry* **2000**, *55*, 727–731.
(20) Qin, S.; Chen, S. H.; Guo, Y. W.; Gu, Y. C. *Helv. Chim. Acta* **2007**, *90*, 2041–2046.
(21) Nishida, T.; Wahlberg, I.; Enzell, C. R. *Org. Magn. Reson.* **1977**, *9*, 203–208.
(22) Mosmman, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
(23) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemarker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.
(24) Zhou, J. J.; Yue, X. F.; Han, J. X.; Yang, W. Y. *Chin J. Pharm.* **1993**, *24*, 455–457.
(25) Reed, L. J.; Muench, H. *Am. J. Hyg.* **1938**, *27*, 493–497.

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