Abiesatrines A–J: anti-inflammatory and antitumor triterpenoids from *Abies georgei* Orr†

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A novel *spiro*-lanostane (abiesatrine A, **1**) was isolated from the aerial parts of *Abies georgei* together with 9 new (abiesatrines B–J, **2–10**) and 10 known triterpenes (**11–20**). The new structures were established by the extensive analysis of their spectroscopic data. The configuration of **1**, featuring a unique spirolactone formed by C-13 and C-23 *via* oxygen-bridge, was confirmed by X-ray crystallography, and its biopathway was tentatively proposed. Among these isolates, compound **16** showed the strongest inhibitory activity against LPS-induced NO production in RAW264.7 macrophages (IC_{50} = 8.9 μg mL^{-1}). While compounds **1** and **20** exhibited potent anti-proliferative effects on QGY-7703 cells with IC_{50} values of 9.3 and 7.6 μg mL^{-1}, respectively. Preliminary structure–activity relationship (SAR) investigations defined structural feature of the 24*Z*-olefinic bond key to the lanostane and cycloartane pharmacophore.

Introduction

Abies georgei occurs exclusively in the northwest of Yunnan Province and the southwest of Sichuan Province, China.[1] The $CHCl_3$ fraction of its EtOH extract showed significant antitumor effect, while the EtOAc part exhibited potent anti-inflammatory activity.[2] In the previous study on this plant, the isolation, structure, and bioactivities of flavanols, diterpenes, and norditerpenes were reported.[3–5] To continuously explore the structurally and biologically novel chemical constituents from *A. georgei*, an intensive investigation was carried out, which led to the isolation of ten new (**1–10**) (Fig. 1) and 10 known (**11–20**) triterpenes. Herein, we describe the isolation, structural elucidation, and anti-inflammatory, as well as antitumor activities of these triterpenes. In addition, preliminary structure–activity relationship (SAR) for lanostanes and cycloartanes on anti-proliferative activity against QGY-7703 tumor cells were investigated.

Results and discussion

The $CHCl_3$- and EtOAc-soluble extracts of *Abies georgei* were subjected to column chromatography (CC) on silica gel, ODS, and Sephadex LH-20, as well as preparative TLC to afford ten new (**1–10**) and ten known (**11–20**) triterpenes. By comparison of the ^1H and ^{13}C NMR, and MS data with the published data, the known compounds were identified as (24*R*)-cycloartane-3β,24,25-triol (**11**),[6,7] (24*R*)-cycloartane-3α,24,25-triol (**12**),[7] methyl (24*Z*)-26-carboxy-3,4-seco-cycloarta-4(29),24-dien-3-oate (**13**),[8] 23-oxo-mariesiic acid B (**14**),[9] isofirmanoic acid (**15**),[9,10] (9β,24*Z*)-3,23-dioxolanosta-7,24-dien-26-oic acid (**16**), firmanoic acid (**17**),[9] 2α,3β,24- trihydroxy-12-ursen-28-oic acid (**18**),[11] dammarolic acid (**19**),[12] and ursolic acid (**20**).[13]

The molecular formula $C_{30}H_{46}O_4$ of compound **1** was established on the basis of its positive HRESIMS at *m/z* 493.3293 $[M+Na]^+$, indicating eight degrees of unsaturation. The IR spectrum showed absorption bands characteristic of hydroxy (3384 cm^{-1}), carboxyl (1773 cm^{-1}) and olefinic bonds (1646 cm^{-1}). The ^1H and ^{13}C NMR spectroscopic data of **1** (Tables 1 and 2) indicated thirty carbon signals including five singlet and two doublet methyls [δ_H 0.89 (3H, d, J = 6.0 Hz, Me-21), 0.90 (3H, s, Me-19), 0.91 (3H, s, Me-29), 0.95 (3H, s, Me-28), 0.98 (3H, s, Me-18), 1.13 (3H, s, Me-30), 1.32 (3H, d, J = 7.2 Hz, Me-26); δ_C 15.7 (q, Me-21), 16.0 (q, Me-26), 17.3 (q, Me-18), 21.7 (q, Me-30), 21.8 (q, Me-19), 22.5 (q, Me-29), 27.8 (q, Me-28)], nine methylenes, six methines (one olefinic carbon and one oxygenated sp^3 methines), and eight quaternary carbons including one carboxyl (δ_C 179.0), one sp^2 carbon (δ_C 143.0), and two oxygenated sp^3 quaternary carbons (δ_C 90.9 and 109.0). In the ^1H–^1H COSY spectrum, six fragments were obtained according to the spin systems of H_2-1/H_2-2/H-3, H-5/H_2-6/H-7, H-9/H_2-11/H_2-12, H_2-15/H_2-16, H_3-21/H-20/H_2-22, and H_2-24/H-25/H_3-26 (Fig. 2). According to the correlations traced from seven methyls (Me-18,19,21,26,28,29,30) and the olefinic proton (δ_H 5.48, dd, J = 5.4, 2.4 Hz, H-7), the structure of 13,17-*friedo*-lanostane triterpenoid was established for compound **1**. According to the unsaturation degrees (Ω = 8) and signals for a hemiketal moiety (δ_C 109.0, C-23) together with an oxygenerated quaternary carbon (δ_C 90.9, C-13), the planar structure of **1** was supposed to contain a novel spirolactone moiety. Further

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† Electronic supplementary information (ESI) available: The MS, ^1H, and ^{13}C NMR data for compounds **11–20**, X-ray crystal data, 1D, and 2D NMR spectra for compound **1**. CCDC 761403. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c001885f

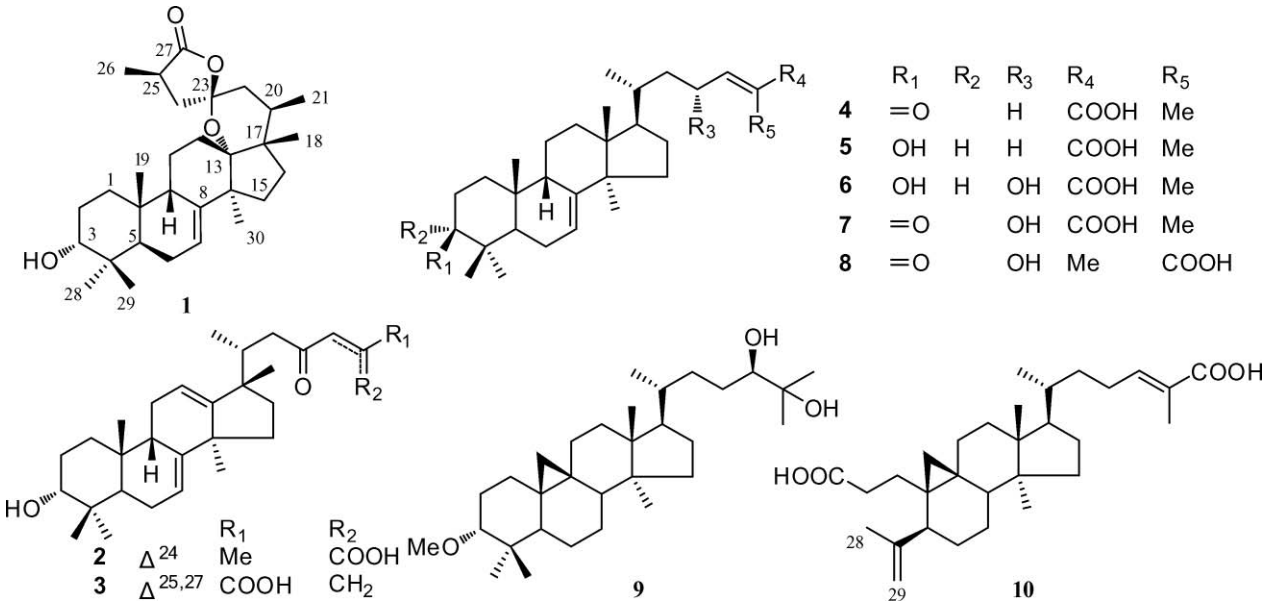


Fig. 1 Structures of compounds **1–10**.

evidence for its relative configuration was confirmed undoubtedly by the X-ray diffraction analysis‡ (Fig. 3). Therefore, **1** was elucidated as 13,17-*friedo*-13,23-epoxy-3α-hydroxy-9β-lanosta-7-ene-23,27-olide, named abiesatrine A. It is the first example of lanostane triterpenoid bearing a unique spirolactone formed by C-13 and C-23 *via* oxygen-bridge. A tentative biosynthetic pathway is proposed in Scheme 1. It might be synthesized from **2** *via* hydration reaction and aldol condensation to give 13,17-*friedo*-13,23-epoxy-3α,23-dihydroxy-9β-lanosta-7,24-diene-27-oic acid. Then intramolecular lactonization of the intermediate followed by reduction constructed the spirolactone system of abiesatrine A.

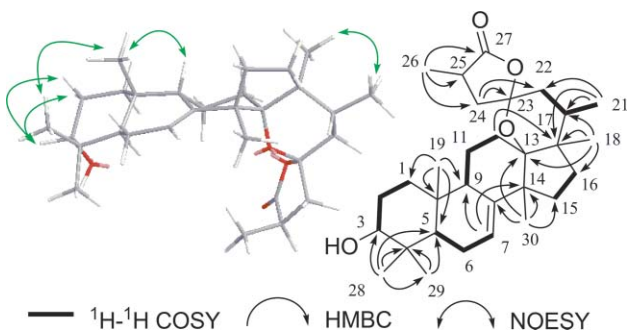


Fig. 2 Key ^{1}H–^{1}H COSY, HMBC, and NOESY correlations for **1**.

‡ X-Ray diffraction analysis of abiesatrine A (1): colorless orthorhombic crystal of $C_{30}H_{46}O_4 \cdot CH_3OH$. Space group $P1$, a = 8.165(15) Å, b = 13.02(3) Å, c = 26.83(6) Å, V = 2852(10) Å^3, Z = 4; crystal size 0.15 × 0.10 × 0.04 mm^3. A total of 11 872 unique reflections (θ = 1.52–25.01°) were collected using graphite monochromated Mo-Kα (λ = 0.71073 Å) on a CCD area detector diffractometer. The structure was solved by direct methods (SHELXS-97) and expanded using Fourier techniques (SHELXS-97). The final cycle of full-matrix least-squares refinement was based on 5047 data, 0 restraints and 327 variable parameters. Final R indicates R_1 = 0.0711, wR_2 = 0.1422 [$I > 2\sigma(I)$]. Crystallographic data (excluding structure factors) for the structure of abiesatrine A (**1**) in this paper are available as ESI.†

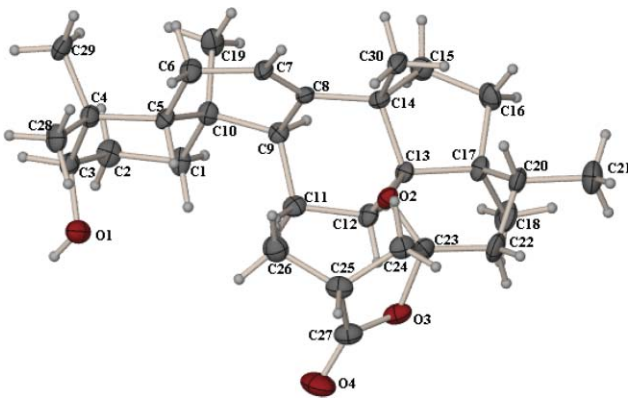


Fig. 3 X-Ray structure of abiesatrine A (**1**). Displacement ellipsoids are drawn at the 50% probability level.

Compound **2** exhibited the molecular formula $C_{30}H_{44}O_4$ as evidenced by the positive HRESIMS at m/z 491.3123 [M+Na]$^+$. It showed ^{1}H and ^{13}C NMR spectra similar to those of **14**. However, close comparison of the ^{13}C NMR spectroscopic data between **2** and **14** revealed significant differences: C-24 was shifted downfield by 2.6 ppm, while C-25 upfield 1.5 ppm. Such phenomena were also found in other *E*/*Z*-configuration pairs of the lanostanes.[9] Thus, the configuration of the olefinic bond at C-24 in **2** should be *Z* compared to *E* in **14**. In the NOESY spectrum, H-24 was correlated to H_3-27 (Fig. 4), which confirmed the presence of *Z*-orientated olefinic bonds at C-24 in **2**. On the basis of above evidences, compound **2** was then established as 13,17-*friedo*-3α-hydroxy-9β-lanosta-7,12,24*Z*-triene-23-oxo-27-oic acid, named abiesatrine B.

Compound **3** shared the same molecular formula as **2**. And they exhibited similar IR and NMR data. However, differences were found by careful comparison of their ^{1}H and ^{13}C NMR spectroscopic data: the methyl at C-26 and vinyl methine at C-24 in **2** were altered to be one olefinic [δ_H 6.04 (1H, d, J = 2.1 Hz), 5.37 (1H, d, J = 2.1 Hz); δ_C 124.4 t] and one aliphatic [δ_H 3.51 (2H,

Table 1 ^{1}H NMR spectroscopic data for compounds **1–10** (*J* in Hz within parentheses)

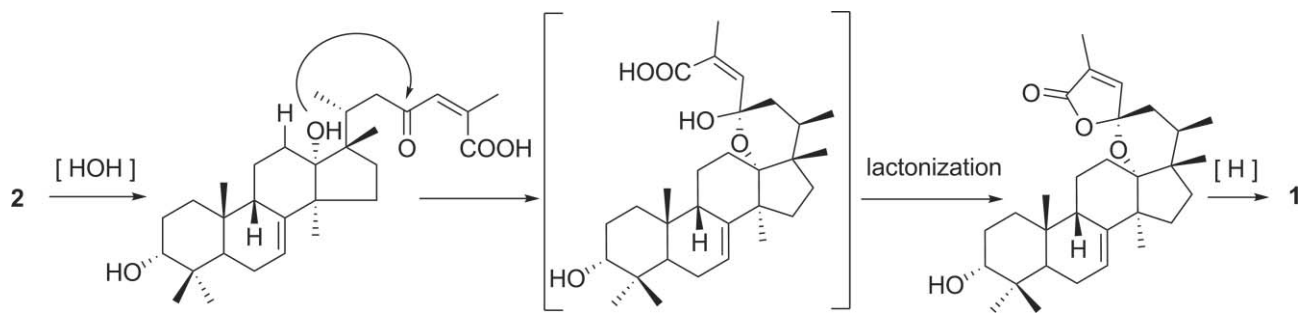
No.	**1**[a]	**2**[b]	**3**[b]	**4**[a]	**5**[a]	**6**[c]	**7**[b]	**8**[c]	**9**[b]	**10**[b]
1	1.97 m; 0.84 m	2.28 m; 1.83 m	1.28 brs; 0.95 m	1.73 m; 1.61 m	1.80 m; 1.41 m	1.88 m; 1.43 m	2.52 m; 1.60 m	2.52 m; 1.75 m	2.13 m; 1.90 m	2.48 m; 2.00 m
2	1.60 m; 1.35 m	1.95 m; 1.58 m	2.01 m; 1.57 m	2.50 (dt, 7.5, 1.8)	1.64 m	1.66 m; 1.59 m	2.44 m; 1.57 m	2.46 m; 1.63 m	1.61 m; 1.57 m	2.43 m; 2.18 m
3	3.44 (t, 3.0)	3.39 m	3.38 m		3.22 (dd, 10.2, 5.4)	3.12 (dd, 11.4, 4.5)			2.87 (t, 3.0)	
5	1.73 m	1.45 m	1.42 m	1.42 (dt, 12.0, 1.2)	0.85 (dd, 10.8, 3.6)	0.87 (dd, 12.0, 4.2)	1.45 m	1.46 m	1.81 (dd, 12.0, 4.8)	2.51 (dd, 12.0, 5.4)
6	1.94 m	1.92 m	1.92 m	1.94 m; 1.89 m	1.94 m	1.92 m	1.92 m; 1.79 m	1.93 m; 1.79 m	1.54 m; 0.79 m	1.48 m
7	5.48 (dd, 5.4, 2.4)	5.66 m	5.66 m	5.65 (dt, 7.8, 2.7)	5.57 (dt, 7.2, 3.0)	5.58 (dt, 7.2, 1.8)	5.66 (dt, 8.1, 2.8)	5.67 (dt, 7.8, 3.0)	1.58 m; 1.03 m	2.08 m; 1.30 m
8									1.59 m	1.59 m
9	1.60 m	1.42 m	1.45 m	2.21 m	2.27 m	2.29 (brd, 13.2)	2.24 m	2.25 m		
11	1.71 m	2.25 m	2.34 m	1.64 m	1.91 m	1.75 m	1.66 m	1.66 m	2.03 m	2.23 m
	1.35 m	1.81 (dd, 14.7, 2.4)	1.82 m		1.69 m	1.45 m			1.06 m	1.33 m
12	1.95 m; 1.79 m	5.56 (dd, 8.4, 2.4)	5.55 (dd, 8.1, 2.4)	1.85 m; 1.72 m	1.56 m; 1.16 m	1.82 m; 1.68 m	1.64 m	1.87 m; 1.66 m	1.63 m	1.68 m; 1.58 m
15	1.93 m; 1.15 m	1.45 m	1.53 m; 1.42 m	1.60 m; 1.43 m	1.60 m; 1.51 m	1.49 m; 1.23 m	1.55 m; 1.42 m	1.58 m; 1.44 m	1.31 m	1.32 m
16	1.92 m	1.92 m	1.91 m	1.96 m	1.95 m	2.00 m	2.05 m	2.04 m	1.31 m	1.32 m
	1.54 m	1.44 m	1.43 m	1.29 m	1.26 m	1.52 m	1.28 m	1.26 m	1.09 m	
17				1.54 m	1.46 m	1.52 m	1.56 m	1.58 m	1.63 m	1.64 m
18	0.98 s	0.96 s	0.94 s	0.79 s	0.91 s	0.89 s	0.77 s	0.77 s	1.00 s	0.99 s
19	0.90 s	0.95 s	0.95 s	1.00 s	0.98 s	0.99 s	0.98 s	0.99 s	0.52 (d, 4.2)	0.73 (d, 4.2)
									0.34 (d, 4.2)	0.41 (d, 4.2)
20	1.80 m	2.21 m	2.20 m	1.42 m	1.42 m	1.33 m	1.35 m	1.31 m	1.38 m	1.43 m
21	0.89 (d, 6.0)	0.88 (d, 6.3)	0.87 (d, 6.3)	0.92 (d, 6.6)	0.91 (d,6.6)	0.95 (d, 6.6)	0.96 d (d, 6.3)	0.97 (d, 6.0)	0.91 (d, 6.0)	0.94 (d, 6.0)
22	1.77 m	2.92 (dd, 14.1, 1.8)	2.86 (dd, 16.0, 2.7)	1.63 m	1.43 m	1.63 m	1.63 m	1.64 m	1.01 m	1.32 m
	1.73 (d, 10.2)	2.23 m	2.34 (d, 16.0)	1.58 m	1.30 m	1.39 m	1.38 m	1.41 m		1.18 m
23				2.24 m; 2.15 m	2.25 m; 2.11 m	4.51 (dt, 9.6, 4.5)	4.50 (dt, 9.3, 4.5)	4.52 (dt, 9.6, 4.8)	1.90 m; 1.31 m	2.20 m; 2.08 m
24	2.29 (dd, 12.6, 9.0)	6.86 (d, 1.5)	3.51 s	6.91 (dt, 7.5, 1.2)	6.90 (t, 7.2)	6.55 (dd, 9.6, 1.2)	6.45 (dd, 9.3, 1.2)	6.56 (dd, 9.0, 1.2)	3.16 (dd, 10.2, 1.8)	6.64 (t, 7.8)
	2.00 (dd, 12.6, 7.2)									
25	2.66 m									
26	1.32 (d, 7.2)	2.16 (d, 1.5)						1.86 (d, 1.2)	1.12 s	
27			6.04 (d, 2.1)	1.85 br s	1.85 s	1.86 (d, 1.2)	1.86 (d, 1.2)		1.15 s	1.80 s
			5.37 (d, 2.1)							
28	0.95 s	0.94 s	0.94 s	1.10 s	1.02 s	0.98 s	1.07 s	1.08 s	0.91 s	1.69 s
29	0.91 s	0.92 s	0.93 s	1.11 s	0.88 s	0.85 s	1.08 s	1.09 s	0.89 s	4.84 (d, 2.4)
										4.72 (dd, 2.4, 1.2)
30	1.13 s	1.20 s	1.18 s	1.03 s	1.03 s	1.05 q	1.05 s	1.06 s	0.94 s	1.01 s
OMe									3.31 s	

[a] Measured at 600 MHz in $CDCl_3$. [b] Measured at 300 MHz in CD_3OD. [c] Measured at 600 MHz in CD_3OD

Table 2 ^{13}C NMR spectroscopic data for compounds **1–10**

No.	**1**[a]	**2**[b]	**3**[b]	**4**[a]	**5**[a]	**6**[c]	**7**[b]	**8**[c]	**9**[b]	**10**[b]
1	28.1 t	30.7 t	30.7 t	34.2 t	35.2 t	36.9 t	35.2 t	35.2 t	28.7 t	31.2 t
2	25.2 t	26.5 t	26.5 t	34.3 t	27.9 t	28.6 t	35.3 t	35.3 t	29.1 t	34.4 t
3	76.6 d	77.2 d	77.2 d	219.1 s	79.4 d	80.0 d	221.5 s	221.7 s	88.0 d	180.4 s
4	36.9 s	38.0 s	38.0 s	47.0 s	38.8 s	39.9 s	48.1 s	48.1 s	40.9 s	150.9 s
5	36.5 d	39.4 d	39.4 d	52.4 d	48.6 d	50.0 d	53.7 d	53.7 d	42.9 d	47.0 d
6	23.8 t	24.3 t	24.3 t	23.0 t	23.1 t	24.2 t	23.9 t	23.9 t	22.0 t	29.1 t
7	118.4 d	120.1 d	120.0 d	121.5 d	121.5 d	122.9 d	122.7 d	122.8 d	29.2 t	26.2 t
8	143.0 s	147.5 s	147.6 s	148.7 s	148.8 s	150.1 s	149.9 s	150.0 s	49.4 d	49.6 d
9	48.3 d	52.6 d	52.7 d	45.5 d	48.4 d	49.8 d	46.8 d	46.8 d	21.0 s	22.6 s
10	34.8 s	36.0 s	36.0 s	35.8 s	35.9 s	37.0 s	36.9 s	37.0 s	27.8 s	28.6 s
11	25.9 t	29.1 t	29.1 t	20.9 t	22.9 t	24.0 t	21.9 t	21.9 t	26.8 t	28.1 t
12	34.7 t	123.8 d	123.6 d	34.4 t	34.6 t	36.6 t	35.6 t	35.6 t	34.2 t	34.4 t
13	90.9 s	157.4 s	157.4 s	44.0 s	43.6 s	44.8 s	45.1 s	45.2 s	46.4 s	46.4 s
14	53.9 s	51.2 s	51.1 s	51.9 s	52.7 s	54.0 s	53.1 s	53.1 s	50.1 s	50.2 s
15	33.7 t	37.9 t	37.9 t	33.1 t	33.4 t	34.5 t	34.2 t	34.2 t	36.6 t	36.8 t
16	39.3 t	39.2 t	39.1 t	29.7 t	28.6 t	30.0 t	29.6 t	29.6 t	27.4 t	29.2 t
17	45.1 s	47.6 s	47.5 s	53.0 d	53.4 d	55.4 d	55.0 d	54.9 d	53.7 d	53.6 d
18	17.3 q	25.4 q	25.6 q	22.4 q	23.6 q	24.1 q	22.9 q	22.8 q	18.5 q	18.7 q
19	21.8 q	22.8 q	22.8 q	23.1 q	24.5 q	24.9 q	23.5 q	23.5 q	30.6 t	31.0 t
20	34.6 d	40.2 d	39.4 d	36.1 d	36.1 d	34.8 d	34.7 d	34.8 d	37.8 d	37.2 d
21	15.7 q	16.2 q	16.2 q	18.2 q	18.2 q	20.0 q	20.0 q	20.0 q	19.0 q	18.7 q
22	37.8 t	49.4 t	46.9 t	34.6 t	35.5 t	44.7 t	44.8 t	44.7 t	34.9 t	36.3 t
23	109.0 s	205.5 s	212.2 s	26.0 t	26.0 t	67.5 d	67.6 d	67.5 d	30.7 t	26.5 t
24	44.8 t	129.7 d	48.2 t	145.7 d	145.6 d	144.8 d	142.6 d	145.0 d	80.6 d	141.7 d
25	35.3 d	149.1 s	141.8 s	126.6 s	126.6 s	129.9 s	132.1 s	129.6 s	73.9 s	130.7 s
26	16.0 q	15.9 q	173.9 s	172.6 s	172.7 s	171.7 s	173.6 s	13.2 q	25.0 q	174.1 s
27	179.0 s	174.6 s	124.4 t	12.0 q	12.0 q	13.2 q	13.7 q	171.4 s	25.7 q	12.9 q
28	27.8 q	28.9 q	28.9 q	28.0 q	28.9 q	29.5 q	28.4 q	28.4 q	26.4 q	20.2 q
29	22.5 q	23.6 q	23.6 q	21.3 q	16.4 q	17.0 q	21.7 q	22.0 q	22.0 q	112.0 t
30	21.7 q	26.6 q	26.6 q	27.4 q	30.5 q	31.0 q	27.9 q	19.8 q	19.8 q	19.8 q
OMe									57.4 q	

[a] Measured at 150 MHz in $CDCl_3$. [b] Measured at 75 MHz in CD_3OD. [c] Measured at 150 MHz in CD_3OD



Scheme 1 Postulated biosynthetic pathway for abiesatrine A (**1**).

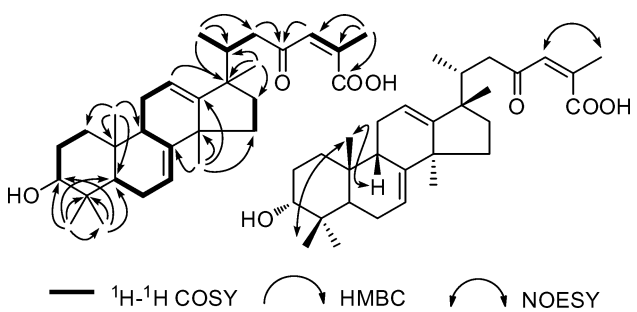


Fig. 4 Selected ^{1}H–^{1}H COSY, HMBC, and NOESY correlations for **2**.

s); δ_C 48.2 t] methylenes in **3**. This suggested that the vinyl bond in **3** should be located at positions of C-25 and C-27, instead of C-24,25 in **2**. By detailed analysis of the HMBC NMR spectrum (Fig. 5), compound **3** was then concluded to be 13,17-*friedo*-3α-hydroxy-9β-lanosta-7,12,25(27)-trien-23-oxo-26-oic acid, named abiesatrine C.

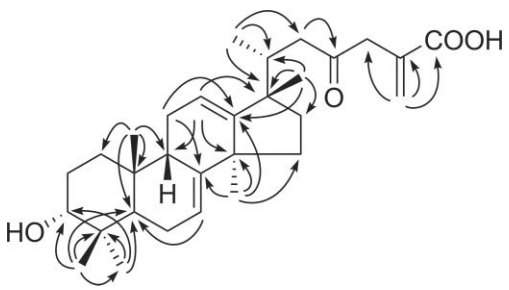


Fig. 5 Key HMBC correlations for compound **3**.

Compound **4** was found to possess the molecular formula $C_{30}H_{46}O_3$, as evidenced by its negative HRESIMS at m/z 453.3320 $[M - H]^-$. The ^1H and ^{13}C NMR spectra showed high similarity to those of (24*E*)-3-oxo-9β-lanosta-7,24-dien-26-ol except for a carboxy group (δ 172.6 s) at C-26 of **4** instead of an oxymethylene moiety in (24*E*)-3-oxo-9β-lanosta-7,24-dien-26-ol.[14] This assumption was confirmed by the HMBC correlations of H_3-27 to the carboxyl (Fig. 6). Therefore, compound **4** was assigned as (24*E*)-3-oxo-9β-lanosta-7,24-dien-26-oic acid, named abiesatrine D

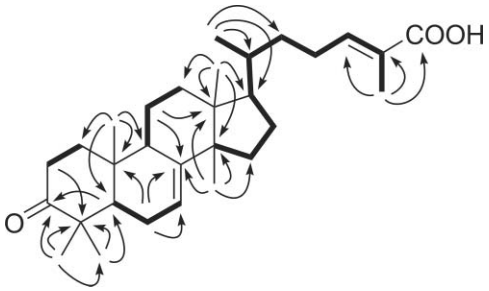


Fig. 6 Key ^1H–^1H COSY and HMBC correlations for compound **4**.

Compound **5** gave the molecular formula $C_{30}H_{48}O_3$ from the negative HRESIMS at m/z 455.3507 $[M - H]^-$. Its ^1H and ^{13}C NMR spectroscopic data were very similar to those of **4** except that an sp^2 ketone group in **4** was changed to be an sp^3 oxymethine [δ_H 3.22 (1H, dd, J = 10.8, 5.4 Hz); δ_C 79.4 d] in **5**. This indicated a hydroxy moiety at C-3 in **5** instead of a ketone in **4**. This assumption was confirmed by the HMBC correlations of H_3-28,29 to the oxymethine at δ_C 79.4 (Fig. 7). According to the large coupling constant (dd, $^3J_{H2-H3}$ = 10.8, 5.4 Hz) of H-2 and H-3, the configuration of 3-OH was deduced as β-orientated. Further evidences were obtained in the NOESY spectrum by the correlations of H-3 to H-1α,2α,5, H_3-28, and H_3-19 to H_3-29, H-9 (Fig. 7). Accordingly, the structure of compound **5** was defined as (24*E*)-3β-hydroxy-9β-lanosta-7,24-dien-26-oic acid, named abiesatrine E.

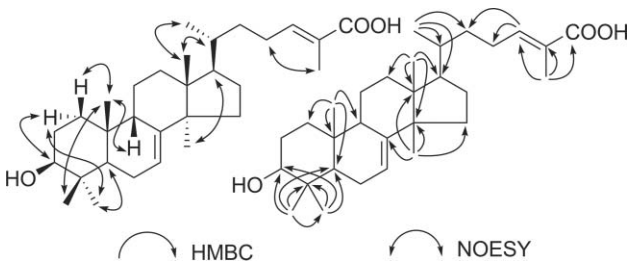


Fig. 7 Selected HMBC and NOESY correlations for compound **5**.

Compound **6** had a molecular formula $C_{30}H_{48}O_4$ as established from its negative HRESIMS at m/z 471.3453 $[M - H]^-$. The ^1H and ^{13}C NMR spectroscopic data were related closely to those of **5**, with the only difference being the presence of an additional hydroxy group at C-23 position [δ_H 4.51 (1H, dt, J = 9.6, 4.5 Hz); δ_C 67.5 d]. This can be confirmed in the ^1H–^1H COSY spectrum by the correlations of vinyl proton (δ_H 6.55, 1H, dd, J = 9.6, 1.2 Hz) to oxymethine (δ_H 4.51, 1H, dt, J = 9.6, 4.5 Hz) and H_3-27 (δ_H 1.86, 3H, d, J = 1.2 Hz) (Fig. 8). The *R*-configuration of C-23 was determined mainly based on the coupling constant (J = 9.6 Hz) of H-23 and H-24.[15] As such, compound **6** was deduced to be (23*R*,24*E*)-3β,23-dihydroxy-9β-lanosta-7,24-dien-26-oic acid, named abiesatrine F.

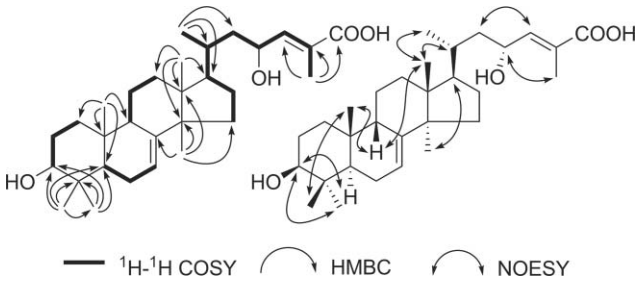


Fig. 8 Selected ^1H–^1H COSY, HMBC and NOESY correlations for compound **6**.

Compound **7** presented a molecular formula of $C_{30}H_{46}O_4$ by positive HRESIMS at m/z 493.3272 $[M+Na]^+$. The ^1H and ^{13}C NMR spectra consisted of signals similar to those of **6** except for a ketone moiety (δ 221.5 s) at C-3 instead of a hydroxy group in **6**. By detailed analysis of its HSQC, ^1H-^1H COSY, HMBC, and NOESY spectra (Fig. 9), **7** was thus assigned as (23*R*,24*E*)-3-oxo-9β-lanosta-7,24-dien-23-hydroxy-26-oic acid, named abiesatrine G.

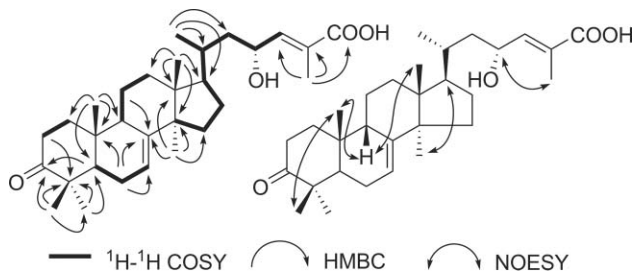


Fig. 9 Key ^1H–^1H COSY, HMBC and NOESY correlations for **7**.

Compound **8** gave the same molecular formula as **7**. In addition, it shared almost the same IR, ^1H and ^{13}C NMR spectroscopic data as those of **7**. However, a close inspection of their ^{13}C NMR spectroscopic data revealed significant differences: downshift of C-24 by 2.4 ppm, while upshift of C-25 by 2.5 ppm. This suggested the presence of a *Z*-oriented vinyl bond at C-24 of **8** instead of *E*-configuration in **7**. The similar variations because of the differences of *E*/*Z*-configuration can be found in compounds **2** and **14**, as well as the other lanostanes.[9] In the NOESY spectrum, H_3-26 was correlated to H-24 (Fig. 10), which confirmed the presence of a 24*Z*-olefinic bond. Consequently, compound **8** was established to

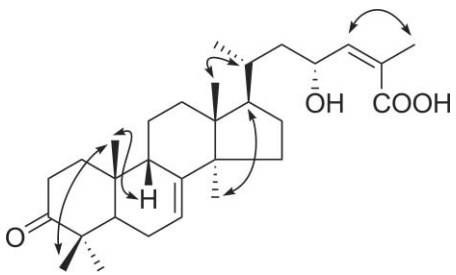


Fig. 10 Selected NOESY correlations for compound **8**.

be (23*R*,24*Z*) 3-oxo-9β-lanosta-7,24-dien-23-hydroxy-27-oic acid, named abiesatrine H.

Compound **9** gave a molecular formula $C_{31}H_{54}O_3$ deduced from its positive HRESIMS at *m/z* 497.4057 $[M+Na]^+$. It exhibited very similar IR, ^1H and ^{13}C NMR spectra to those of **12**[7] except for an additional methoxyl [δ_H 3.31 (3H, s); δ_C 57.4 (q)]. Compared to **12**, C-3 of **9** was upshifted by 10.3 ppm, which established the connection of the methoxyl to C-3 position. By its HMBC correlations (Fig. 11), compound **9** was then identified to be (24*R*)-3α-methoxycycloartane-24,25-diol, named abiesatrine I.

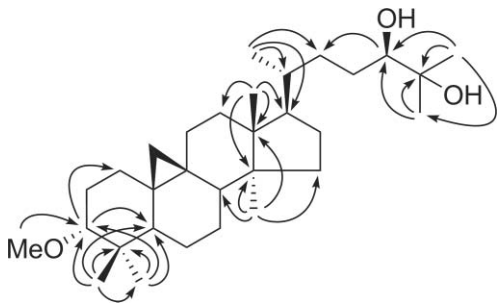


Fig. 11 Key HMBC correlations for compound **9**.

Compound **10** showed a molecular ion peak at *m/z* 469.3321 $[M - H]^-$ in its negative HRESIMS, corresponding to the molecular formula of $C_{30}H_{46}O_4$. Its ^1H and ^{13}C NMR spectroscopic data were very similar to those of **13**[8] except for the presence of a carboxy group instead of methyl ester moiety at C-3, which can be confirmed according to the downshift of C-2 and C-3 by 2.0 and 4.2 ppm, respectively. In addition, C-25 was downshifted by 2.1 ppm, while C-24 upshifted by 2.7 ppm. According to the similar phenomena in **2**/**14**, **7**/**8**, and other triterpenes,[9] compound **10** was supposed to bear an *E*-configuration olefinic bond at C-24,25. By detailed analysis of its 2D NMR spectra (Fig. 12), **10** was then concluded to be (24*E*)-3,4-secocycloarta-4(28),24-dien-3,26-dioic acid, named abiesatrine J.

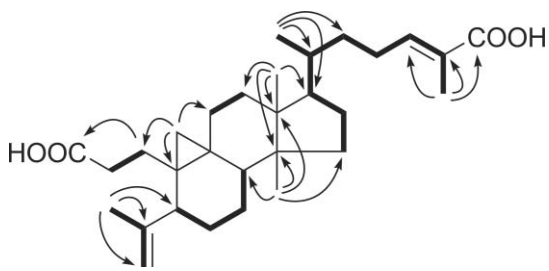


Fig. 12 Key ^1H–^1H COSY (bold) and HMBC (arrow) correlations for compound **10**.

Lanostanes are the main triterpenes in *Abies* plants together with minor cycloartanes, gammacerane, and hopanes.[16,17] This is coincident with the results obtained from this study on *A. georgei*. Briefly, among the isolated 20 triterpenes, 12 are lanostanes (**1–8** and **14–17**) and 5 cycloartanes (**9–13**). The other three are ursanes (**18–20**), which is a new type of triterpenes found in *Abies* species for the first time.

Since the $CHCl_3$ fraction of the EtOH extract of *A. georgei* showed potent antitumor effect against QGY-7703 and LOVO cells, while the EtOAc part exhibited strong inhibitory activity on nitric oxide (NO) induction by lipopolysaccharide (LPS) in RAW264.7 macrophages,[2] all the isolates (**1–20**) were then subjected to these two bioassays. For inhibitory effects against LPS-induced NO production, compound **16** showed remarkably active (IC_{50} = 8.9 μg mL^{-1}), while **15** and **17** exhibited weak activities with IC_{50} values of 19.8 and 17.6 μg mL^{-1}, respectively. For antitumor experiments against QGY-7703 and LOVO cells, compounds **1** and **20** displayed a significant effect with IC_{50} values of 9.3 and 7.6 μg mL^{-1}, respectively. However, only **20** showed moderate activity on LOVO cells (IC_{50} = 14.7 μg mL^{-1}). It is interesting to note that compounds displaying anti-inflammatory activities were all isolated from EtOAc fraction, while those with antitumor activities were from $CHCl_3$ extract (except **16**). This is coincident with the previous bioactive results of the crude extracts of *A. georgei*.[2]

Experimental

General

NMR spectra were recorded on a Bruker Avance 600 or Avance 300 NMR spectrometer with TMS as internal standard. ESIMS were measured on an Agilent LC/MSD Trap XCT spectrometer (Waters, USA), and HRESIMS on a Q-TOF micro mass spectrometer (Waters, USA). Optical rotations were acquired with Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Bruker Vector-22 spectrometer with KBr pellets. Materials for CC were silica gel (Huiyou Silical Gel Development Co. Ltd., Yantai, China), Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-GEL ODS-A (YMC, USA). Prep. TLC was conducted with glass precoated silica gel GF_{254} (Yantai).

Plant material

The aerial parts of *A. georgei* Orr were collected from Zhongdian city, Yunnan Province of China in July 2006, and were identified by Prof. Li-Shang Xie in Kunming Institute of Botany, Chinese Academy of Sciences. A herbarium specimen (No. 2006-07-016) was deposited in School of Pharmacy, Second Military Medical University, China.

Extraction and isolation

The plant material (22 kg) was pulverized and extracted with 80% EtOH under reflux for 3 × 3 h. The extracts were combined to concentrate to a small volume and then partitioned with $CHCl_3$ (25 L), EtOAc (40 L), and n-BuOH (50 L), respectively. The EtOAc extract (282 g) was separated into six fractions (F_1–F_6) by CC over silica gel eluting with gradient $CHCl_3$–Me_2CO. Fraction F_1 (36.3 g) was subjected to CC over MCI, Sephadex LH-20, and silica gel to give **16** (195.2 mg) and **17** (100.4 mg). Fraction F_2 was divided into 20 subfractions ($F_{2\text{-}1}$–$F_{2\text{-}20}$) by RP-MPLC eluting with MeOH–H_2O (5 : 95–100 : 0). Compounds **9** (8.6 mg), **11** (85.4 mg), and **15** (44.2 mg) were obtained after CC over LH-20 ($CHCl_3$–MeOH, 1 : 1 and 0 : 1) followed by repeated prep. TLC with $CHCl_3$–MeOH (20 : 1) from subfractions $F_{2\text{-}3}$, $F_{2\text{-}16}$, and $F_{2\text{-}17}$, respectively. After CC on LH-20 with $CHCl_3$–MeOH (1 : 1) and MeOH, subfraction $F_{2\text{-}20}$ was then subjected to prep. TLC using petroleum ether

(PE)–EtOAc (1 : 1) and $CHCl_3$–MeOH (20 : 1) to give **10** (16.0 mg) and **12** (7.1 mg). Similarly, **3** (20.5 mg) and **7** (35.8 mg) were isolated from fraction F_3. The $CHCl_3$ extract (906 g) was separated into five fractions (F_{C1}–F_{C5}) by CC over silica gel eluting with gradient PE–$CHCl_3$. Compounds **1** (52.6 mg), **4** (16.0 mg), and **5** (9.0 mg) were purified from fraction F_{C2} after repeated CC over LH-20 eluting with $CHCl_3$–MeOH (1 : 1) and MeOH, followed by prep. TLC using $CHCl_3$–MeOH (100 : 1) and/or PE–EtOAc (4 : 1). By the similar procedures, **2** (90.7 mg), **6** (13.5 mg), **8** (213.0 mg), **13** (37.0 mg), and **14** (116.6 mg) were isolated from fraction F_{C4}, and **18** (28.2 mg), **19** (29.2 mg), **20** (21.8 mg) from fraction F_{C5}, respectively.

Abiesatrine A (1). Colorless orthorhombic crystals; $[\alpha]_D^{20}$ –42.1 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε): 216 (4.73); IR (KBr) ν_{max} 3384, 2965, 2928, 2873, 1773, 1646, 1383, 1308, 1203, 1166, 1084, 884 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 471 $[M+H]^+$, 493 $[M+Na]^+$, 963 $[2M+Na]^+$; HRESIMS (positive) $[M+Na]^+$ *m/z* 493.3293, calcd for $C_{30}H_{46}O_4Na$, 493.3294.

Abiesatrine B (2). Amorphous powder; $[\alpha]_D^{20}$ –82.7 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε): 216 (4.08); IR (KBr) ν_{max} 3433, 2965, 2924, 2869, 1626, 1446, 1370, 1063, 986, 832 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 491 $[M+Na]^+$; ESIMS (negative) *m/z* 467 $[M-H]^-$, 935 $[2M-H]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 491.3123, calcd for $C_{30}H_{44}O_4Na$, 491.3137.

Abiesatrine C (3). Amorphous powder; $[\alpha]_D^{20}$ –31.4 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε): 211 (3.92); IR (KBr) ν_{max} 3432, 2965, 2925, 2830, 1606, 1587, 1364, 1064, 985, 832 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 491 $[M+Na]^+$; ESIMS (negative) *m/z* 467 $[M-H]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 491.3156, calcd for $C_{30}H_{44}O_4Na$, 491.3137.

Abiesatrine D (4). Amorphous powder; $[\alpha]_D^{20}$ +30.5 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ε): 215 (4.02); IR (KBr) ν_{max} 3411, 2933, 2877, 1736, 1708, 1645, 1457, 1420, 1383, 1281, 1217, 1104, 815 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 477 $[M+Na]^+$; ESIMS (negative) *m/z* 453 $[M-H]^-$; HRESIMS (negative) $[M-H]^-$ *m/z* 453.3320, calcd for $C_{30}H_{45}O_3$, 453.3369.

Abiesatrine E (5). Amorphous powder; $[\alpha]_D^{20}$ –9.8 (*c* 0.32, MeOH); UV (MeOH) λ_{max} (log ε): 211 (3.91); IR (KBr) ν_{max} 3447, 2969, 2944, 2870, 1738, 1685, 1653, 1456, 1365, 1228, 1217, 1026, 911 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 479 $[M+Na]^+$; ESIMS (negative) *m/z* 455 $[M-H]^-$, 911 $[2M-H]^-$; HRESIMS (negative) $[M-H]^-$ *m/z* 455.3507, calcd for $C_{30}H_{47}O_3$, 455.3525.

Abiesatrine F (6). Amorphous powder; $[\alpha]_D^{20}$ –18.0 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.05); IR (KBr) ν_{max} 3405, 2945, 2927, 2874, 2621, 1698, 1682, 1651, 1463, 1384, 1259, 1216, 1162, 1052, 964 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 495 $[M+Na]^+$; ESIMS (negative) *m/z* 471 $[M-H]^-$, 943 $[2M-H]^-$; HRESIMS (negative) $[M-H]^-$ *m/z* 471.3453, calcd for $C_{30}H_{47}O_4$, 471.3474.

Abiesatrine G (7). Amorphous powder; $[\alpha]_D^{20}$ +54.5 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.17); IR (KBr) ν_{max} 3388, 2975, 2930, 2872, 1707, 1625, 1547, 1463, 1383, 1153, 1054, 817 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 493 $[M+Na]^+$; ESIMS (negative) *m/z* 469 $[M-H]^-$, 939 $[2M-H]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 493.3272, calcd for $C_{30}H_{46}O_4Na$, 493.3294.

Abiesatrine H (8). Amorphous powder; $[\alpha]_D^{20}$ +63.9 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε): 215 (4.12); IR (KBr) ν_{max} 3387, 2973, 2928, 2873, 1741, 1708, 1648, 1563, 1514, 1456, 1384, 1149, 877 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 471 $[M+H]^+$, 493 $[M+Na]^+$, 963 $[2M+Na]^+$; ESIMS (negative) *m/z* 469 $[M-H]^-$, 939 $[2M-H]^-$; HRESIMS (negative) $[M-H]^-$ *m/z* 469.3311, calcd for $C_{30}H_{45}O_4$, 469.3318.

Abiesatrine I (9). Amorphous powder; $[\alpha]_D^{20}$ –6.6 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε): 211 (3.79); IR (KBr) ν_{max} 3446, 2931, 2869, 1700, 1651, 1540, 1457, 1383, 1100 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 497 $[M+Na]^+$, 971 $[2M+Na]^+$; ESIMS (negative) *m/z* 509 $[M+Cl]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 497.4057, calcd for $C_{31}H_{54}O_3Na$, 497.3971.

Abiesatrine J (10). Amorphous powder; $[\alpha]_D^{20}$ +23.0 (*c* 0.36, MeOH); UV (MeOH) λ_{max} (log ε): 215 (4.29); IR (KBr) ν_{max} 3445, 2929, 2870, 1771, 1733, 1646, 1559, 1384, 1277, 889 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 493 $[M+Na]^+$; ESIMS (negative) *m/z* 469 $[M-H]^-$; HRESIMS (negative) $[M-H]^-$ *m/z* 469.3321, calcd for $C_{30}H_{45}O_4$, 469.3318.

Assays for anti-inflammatory and antitumor activities

Experiments were carried out according to the previously reported procedures of inhibitory activities against LPS-induced NO production in RAW264.7 macrophages and antitumor activities on QGY-7703 and LOVO cell lines.[2,3] The cell viability was evaluated by MTT reduction, and the absorbances at 570 nm were read using a microtiter plate reader.

Conclusions

Triterpenoids are the main chemical constituents of *Abies* species. From 19 plants of this genus, about 277 compounds were identified, among which 74 were triterpenes and 36 diterpenes.[17] Interestingly, in our recent research for *Abies* plants indigenous in China, many diterpenoids (including norditerpenoids) were isolated.[3,5,18] Since norditerpenoids are not found before from this genus, it is of important chemotaxonomic significance for the norditerpenoids as the characteristics of the *Abies* plants in China.

A comparison of the cytotoxicity of all the isolates (**1–20**) against QGY-7703 cancer cells were carried out. Among 12 lanostane triterpenoids, abiesatrines B (**2**), H (**8**), and (9β,24*Z*)-3,23-dioxolanosta-7,24-dien-26-oic acid (**16**) displayed positive antiproliferative activities. The other lanostanes (except **1**), however, were negative (Table 3). This highlights the structure–activity relationship (SAR) importance of the 24*Z*-olefinic bond. Such SAR can also be extended to cycloartane triterpenoids (*i.e.* for five cycloartanes, only **13** showed positive effect, while compounds **9–12** were negative). Furthermore, close comparison of the cytotoxicity data for **1**, **2**, **6**, and **8** revealed a pharmacophore bias

Table 3 Antiproliferative activities of 20 triterpenes from *Abies georgei* against QGY-7703 and LOVO tumor cell lines

Compounds	IC_{50}	
	QGY-7703	LOVO
1	9.3	> 20.0
2	15.2	> 20.0
8	20.4	> 20.0
13	14.4	> 20.0
16	21.8	18.2
20	7.6	14.7
Others[a]	> 20.0	> 20.0
Doxorubicin[b]	0.5	0.2

[a] Other compounds, including **3–7**, **9–12**, **14**, **15**, **17–19**. [b] Positive control.

in favor of 3α-hydroxylation. These assessments of the lanostane and cycloartane pharmacophore, though only preliminary, do point to clear relationships between structure and potency, and encourage the view that this unusual pharmacophore deserves further investigation.

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