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Abiesanordines A–N: fourteen new norditerpenes from *Abies georgei*

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Abstract

Fourteen new norditerpenoids (abiesanordines A–N, **1**–**14**), including a novel podocarpene bearing a rare enolic structure (abiesanordine A, **1**), were isolated from *Abies georgei* together with eight known ones. Their structures were determined mainly by detailed analysis of 1D and 2D NMR spectroscopic data including HSQC, DQF COSY, HMBC, and NOESY. All the isolates were tested for inhibitory activities against LPS-induced NO production in RAW264.7 macrophages, abiesanordine I (**9**) showed the strongest activity with the IC_{50} value of 17.0 μg/mL. Furthermore, it exhibited no cytotoxicity against RAW264.7 macrophages under the concentration of 50 μg/mL.

Keywords: *Abies georgei*; Pinaceae; Norditerpenoids; Podocarpenes; Abiesanordines A–N; Nitric oxide (NO); RAW264.7 macrophages

1. Introduction

Abies georgei Orr are arbores occurring exclusively in northwest of Yunnan and southwest of Sichuan Provinces, China.[1] Although no evidence was found for the use of this plant in traditional medicine system, the diverse bioactivities and various constituents reported for other *Abies* species [2] stimulated us to carry out the pharmacological and phytochemical investigations on *A. georgei*. Previously, we reported a novel biflavanol isolated from this plant with unique six connective hexacyclic rings by cyclization on C3–*O*–C5′, C4–C4′, and C3′–*O*–C5.[3] Further research, however, resulted in the isolation of 14 new (abiesanordines A–N, **1**–**14**) and 8 known norditerpenes (**15**–**22**). Abiesanordine A (**1**) is the first example of podocarpene bearing a rare enolic structure. Herein, we reported the isolation and structural elucidation of 14 new norditerpenes from *A. georgei*. In addition, the inhibitory activity of all 22 isolates against LPS-induced NO production in RAW264.7 macrophages and the cytotoxicity of 7 bioactive compounds against RAW264.7 macrophages were also described in this paper.

2. Results and discussion

The EtOAc fraction of the EtOH extract of the aerial parts of *A. georgei* was subjected to column chromatography on silica gel, RP-18, and Sephadex LH-20, as well as preparative TLC to afford 14 new (abiesanordines A–N, **1**–**14**) and 8 known norditerpenes: 7α-hydroxypodocarpen-8(14)-en-13-one (**15**),[4] 17-nor-7,15-dion-8,11,13-abietatrien-18-oic acid (**16**),[5] 8(14)-podocarpen-13-on-18-oic acid (**17**),[6] 8(14)-podocarpen-7,13-dion-18-oic acid (**18**),[6] 17-nor-15-oxo-8,11,13-abietatrien-18-oic acid (**19**),[7] 18-nor-abieta-8,11,13-triene-4,15-diol (**20**),[8] 4-hydroxy-18-nor-8,11,13-abietatrien-7-one (**21**),[9] and 8-hydroxy-14,15-dinor-11-labden-13-one (**22**).[10]

Compound **1** gave the molecular formula of $C_{21}H_{26}O_7$ in the positive HRESIMS at *m*/*z* 413.1567 $[M+Na]^+$, indicating nine degrees of unsaturation. The IR spectrum showed bands

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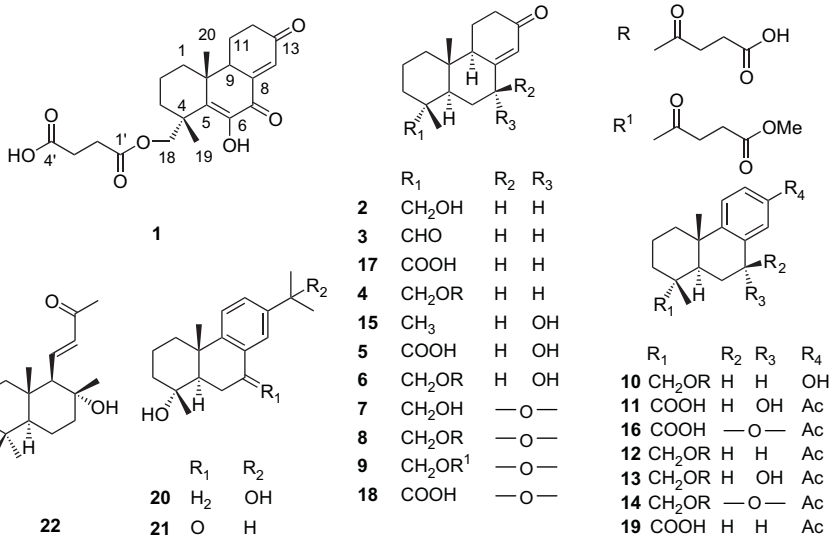

characteristic of hydroxyl (3384 cm^{-1}), carbonyl (1738 and 1710 cm^{-1}), and olefinic bond (1646 and 1588 cm^{-1}). The ^1H and ^{13}C NMR spectroscopic data of **1** (Tables 1 and 2) indicated 21 carbon signals including 2 quaternary methyl singlets [δ_H 1.31 (3H, s, Me-19), 1.16 (3H, s, Me-20); δ_C 22.7 (C-19), 27.7 (C-20)], 2 methines [δ_H 3.02 (dt, J=11.4, 3.0 Hz, H-9), 6.77 (dd, J=3.0, 1.2 Hz, H-14); δ_C 48.7 (C-9), 130.5 (C-14)], 8 methylenes, and 9 quaternary carbons. Since six of the nine degrees of unsaturation were attributed to four carbonyls [δ_C 183.1 (C-7), 202.1 (C-13), 174.2 (C-1′), and 175.8 (C-4′)] and two vinyl groups [δ_C 145.2 (C-5), 147.4 (C-6), 153.0 (C-8), and 130.5 (C-14)], compound **1** was assumed to contain a tricyclic nucleus. In the DQF COSY experiment, the correlations of H-2′ to H-3′, H-1 through H-2 to H-3, and H-12 through H-11 to H-9 and H-14 established three fragments (Fig. 1a). The HMBC correlations traced from the methyls (Me-19,20) and olefinic proton (H-14) suggested the presence of a novel enolic podocarpene diterpenoid moiety (Fig. 1a). However, this could not be readily confirmed because no correlation was found for C-6 of the podocarpene group. As such, the deuterated solvent of MeOH-d_4 was changed to DMSO-d_6 for another HMBC experiment. Fortunately, the correlations from the proton at 6-OH (δ_H 8.33) to C-5 (δ_C 136.0), C-6 (δ_C 144.2), and C-7 (δ_C 179.1) were observed (Fig. 1b), which confirmed unambiguously the existence of the enolic podocarpene diterpene. In addition, a butanedioyl moiety was found according to the DQF COSY experiment and HMBC correlations of two methylenes (H_2-2′,3′). These two groups can be connected as shown in Figure 1a according to the HMBC correlation of H_2-18 with the ester carbonyl at C-1′ of the butanedioyl moiety. The relative stereochemistry of **1** was established mainly by NOESY correlations of H-9 with H-1α, H-11α, and Me-20 with H-1β, Me-19, H-11β (Fig. 1c). In addition, the axial–axial, axial–equatorial, and an allyl coupling of H-9 [δ_H 3.02 (1H, dt, J=11.4, 3.0 Hz)] to H_2-11 and H-14 was in agreement with α-orientation of H-9. Therefore, compound **1** was elucidated as 15-*O*-butanedioylpodocarpen-5,8(14)-dien-6-hydroxy-7,13-dione, named abiesanordine A. This is the first example of podocarpene bearing an enolic structure.

Compound **2** exhibited a $[M+Na]^+$ ion peak at *m/z* 285.1811 in the positive HRESIMS, corresponding to the molecular formula of $C_{17}H_{26}O_2$, which indicated five degrees of unsaturation. Its IR spectrum showed absorbances consistent with hydroxyl (3433 cm^{-1}), carbonyl (1726 cm^{-1}) and olefinic (1660 cm^{-1}) groups. The ^1H NMR spectrum revealed the presence of one vinylic proton [δ_H 5.85 (1H, t, J=2.1 Hz, H-14)], one oxygenated methylene [δ_H 3.39, 3.01 (each 1H, d, J=11.1 Hz, H-18a,b)], and two singlet methyls [δ_H 0.81 (3H, s, Me-19), 0.87 (3H, s, Me-20)]. Besides 5 carbon signals' resonances in the ^1H NMR spectrum, its ^{13}C NMR spectrum exhibited the other 12 signals including a carbonyl (δ_C 202.8). In the DQF COSY experiment, correlations were found from H-1 through H-2 to H-3, from H-12 through H-11 to H-9, and from H-5 through H-6 to H-7. Besides, allyl correlations were also found for the olefinic proton H-14 [δ_H 5.85 (1H, t, J=2.1 Hz)] to H-7,9. Therefore, the DQF COSY experiment established two fragments, which can be connected as shown in Figure 1d on the base of the HMBC correlations originated from the methyls (Me-19,20) and olefinic proton (H-14). The relative configuration was determined by the ROESY correlations of Me-19/Me-20, H-7/H-5, and H-5/H_2-18. Thus, compound **2** was established as 18-hydroxypodocarpen-8(14)-en-13-one, named abiesanordine B.

Compound **3** was assigned the molecular formula of $C_{17}H_{24}O_2$, as established from its HRESIMS at *m/z* 261.1860 $[M+H]^+$, accounting for six degrees of unsaturation. Absorption of carbonyl (1724 and 1709 cm^{-1}) and olefinic bond (1662 cm^{-1}) were observed in its IR spectrum. The ^1H NMR spectrum indicated the presence of one vinylic proton [δ_H 5.87 (1H, t, J=2.1 Hz, H-14)] and two methyls [δ_H 0.90 (3H, s, H-20), 1.11 (3H, s, H-19)], which were similar to those of **2**. In addition, one aldehyde proton [δ_H 9.27 (1H, s, H-18)] was also found in its ^1H NMR spectrum. Compared to abiesanordine B (**2**), **3** was consequently determined as 8(14)-podocarpen-18-al-13-one, named abiesanordine C.

Table 1
^{1}H NMR spectroscopic data for compounds **1**–**16** in CD_3OD (*J* in Hz within parentheses)

No.	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]
1	1.82 m; 1.43 (dt, 13.2, 4.2)	1.80 m; 1.15 m	1.84 m; 1.28 m	1.80 m; 1.22 m	1.79 m; 1.28 m
2	1.69 m	1.57 m	1.67 m	1.55 m	1.60 m
3	1.81 (dt, 13.2, 4.2); 1.52 m	1.57 m; 1.26 m	1.52 m; 1.31 m	1.57 m; 1.40 m	1.89 m; 1.61 m
5		1.64 m	1.89 m	1.53 m	2.53 (dd, 13.2, 3.0)
6		1.72 m; 1.50 (dd, 12.0, 5.4)	1.70 m; 1.55 m	1.72 m; 1.58 m	1.82 m; 1.56 m
7		2.54 (dd, 10.5, 2.1); 2.41 m	2.52 (dt, 10.5, 2.1); 2.38 m	2.35 m	4.27 (t, 3.0)
9	3.02 (dt, 11.4, 3.0)	2.23 m	2.26 m	2.29 m	2.62 (dt, 6.0, 1.2)
11	2.26 m; 1.90 m	2.04 m; 1.72 m	2.08 m, 1.75 m	2.05 m	2.05 m; 1.79 m
12	2.48 m	2.29 m	2.28 m	2.50 m	2.37 m, 2.31 m
14	6.77 (dd, 3.0, 1.2)	5.85 (t, 2.1)	5.87 (t, 2.1)	5.84 (t, 2.1)	5.94 (d, 2.4)
16					
18	4.92 (d, 10.2); 3.98 (d, 10.2)	3.39 (d, 11.1); 3.01 (d, 11.1)	9.27 s	4.10 (d, 11.0); 3.55 (d, 11.0)	
19	1.31 s	0.81 s	1.11 s	0.91 s	1.19 s
20	1.16 s	0.87 s	0.90 s	0.87 s	0.86 s
2′	2.54 m			2.60 m	
3′	2.55 m			2.61 m	
4′-OMe					

No.	**6**[b]	**7**[b]	**8**[b]	**9**[b]	**10**[a]	**11**[b]
1	1.78 m; 1.20 m	1.76 m; 1.18 m	1.85 m; 1.26 m	1.86 m; 1.31 m	2.26 (br d, 12.6); 1.30 m	2.40 (br d, 12.6); 1.51 (dt, 12.6, 4.2)
2	1.58 m	1.57 m	1.64 m; 1.55 m	1.64 m; 1.52 m	1.68 m; 1.61 m	1.86 m; 1.73 m
3	1.48 m; 1.42 m	1.30 m; 1.24 m	1.45 m	1.48 m	1.44 m	1.90 m; 1.65 m
5	2.04 m	2.04 m	2.00 (dd, 13.8, 4.2)	2.04 (dd, 13.2, 5.4)	1.58 (dd, 12.0, 2.7)	2.54 (dd, 13.2, 2.4)
6	1.75 m; 1.28 m	2.37 m; 2.26 m	2.52 m; 2.35 m	2.61 m; 2.52 m	1.75 m; 1.57 (dd, 12.0, 2.4)	2.15 (dt, 14.4, 4.8); 1.70 (br d, 14.4)
7	4.29 (t, 2.7)				2.76 m	4.79 (dd, 4.8, 1.5)
9	2.59 m	2.54 m	2.62 m	2.62 (dd, 3.9, 1.8)		
11	2.07 m; 1.78 m	2.04 m; 1.75 m	2.19 m; 1.76 m	2.21 m; 1.74 m	7.04 (d, 8.4)	7.44 (d, 8.4)
12	2.35 m, 2.29 m	2.48 m; 2.42 m	2.45 m; 2.36 m	2.43 m; 2.38 m	6.52 (dd, 8.4, 2.4)	7.85 (dd, 8.4, 2.1)
14	5.94 (br d, 2.1)	6.52 (dd, 3.0, 1.2)	6.52 br s	6.52 (dd, 3.3, 1.2)	6.42 (d, 2.4)	7.98 (d, 2.1)
16						2.57 s
18	3.93 (d, 11.0); 3.68 (d, 11.0)	3.33 (d, 11.1); 3.02 (d, 11.1)	3.98 (d, 11.1); 3.61 (d, 11.1)	4.07 (d, 11.2); 3.55 (d, 11.2)	4.00 (d, 10.8); 3.71 (d, 10.8)	
19	0.91 s	0.94 s	0.97 s	0.99 s	0.94	1.27 s
20	0.87 s	0.87 s	0.93 s	0.94 s	1.18 s	1.16 s
2′	2.60 m		2.61 m	2.62 m	2.54 m	
3′	2.62 m		2.60 m	2.61 m	2.56 m	
4′-OMe				3.60 s		

No.	**12**[b]	**13**[b]	**14**[b]	**15**[a]	**16**[b]
1	2.37 (dt, 13.5, 3.6); 1.37 m	2.38 (dt, 12.6, 3.0); 1.41 m	2.48 m; 1.58 m	1.75 m; 1.16 m	2.48 m; 1.61 (dt, 6.9, 3.0)
2	1.68 m	1.70 m	1.90 m	1.51 m	1.81 m
3	1.45 m	1.50 m	1.52 m	1.46 m; 1.25 m	1.80 m
5	1.63 m	2.07 m	2.20 (d, 14.1)	1.70 m	2.68 (dd, 14.2, 3.0)
6	1.80 m	2.02 m; 1.90 m	2.77 (d, 14.1)	1.89 m; 1.71 m	2.86 (dd, 17.6, 14.2); 2.42 (dd, 17.6, 3.0)
7	2.94 m	4.82 (t, 2.4)		4.30 (t, 3.0)	
9				2.51 m	
11	7.39 (d, 8.4)	7.45 (8.4)	7.64 (d, 8.4)	2.03 m; 1.75 m	7.63 (d, 8.4)
12	7.71 (dd, 8.4, 2.1)	7.85 (dd, 8.4, 2.1)	8.18 (dd, 8.4, 2.1)	2.32 m; 2.06 m	8.18 (dd, 8.4, 2.1)
14	7.66 (d, 2.1)	7.96 (d, 2.1)	8.51 (d, 2.1)	5.94 (d, 1.8)	8.52 (d, 2.1)
16	2.55 s	2.58 s	2.62 s		2.61 s
18	4.04 (d, 10.8); 3.71 (d, 10.8)	4.02 (d, 10.8); 3.74 (d, 10.8)	3.94 (d, 11.1); 3.74 (d, 11.1)	0.93 s	
19	0.98 s	0.99 s	1.07 s	0.89 s	1.35 s
20	1.24 s	1.18 s	1.31 s	0.83 s	1.32 s
2′	2.56 m	2.57 m	2.51 m		
3′	2.49 m	2.59 m	2.40 m		
4′-OMe					

[a] Recorded at 600 MHz.
[b] Recorded at 300 MHz.

Table 2
^{13}C NMR spectroscopic data for compounds **1**−**22** in CD_3OD

No.	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**8**[a]	**9**[a]	**10**[b]	**11**[a]	**12**[a]	**13**[a]	**14**[a]	**15**[b]	**16**[a]	**17**[a]	**18**[a]	**19**[a]	**20**[a]	**21**[a]	**22**[a]
1	37.4 t	40.0 t	39.2 t	39.7 t	39.4 t	39.7 t	39.4 t	39.0 t	39.2 t	39.8 t	38.7 t	39.3 t	39.0 t	38.4 t	40.3 t	38.2 t	39.6 t	39.7 t	39.2 t	39.4 t	38.5 t	42.2 t
2	18.1 t	19.2 t	18.2 t	19.0 t	19.0 t	19.1 t	18.9 t	18.7 t	18.7 t	19.6 t	19.6 t	19.5 t	19.5 t	19.0 t	19.9 t	19.1 t	19.2 t	18.7 t	19.9 t	21.5 t	21.1 t	19.4 t
3	37.7 t	36.3 t	33.4 t	36.3 t	37.9 t	37.0 t	36.2 t	36.6 t	36.7 t	36.7 t	37.6 t	36.5 t	36.6 t	36.3 t	43.0 t	37.8 t	38.3 t	38.0 t	38.2 t	43.4 t	43.1 t	43.0 t
4	40.2 s	38.9 s	50.6 s	38.1 s	47.8 s	37.6 s	37.0 s	38.0 s	38.0 s	37.9 s	48.1 s	38.1 s	39.7 s	37.9 s	33.9 s	47.5 s	48.3 s	47.0 s	48.1 s	73.2 s	72.2 s	33.9 s
5	145.2 s	47.5 d	46.6 d	47.9 d	42.9 d	42.0 d	43.6 d	44.2 d	44.1 d	46.1 d	40.5 d	45.4 d	39.8 d	44.4 d	47.8 d	45.0 d	49.4 d	45.2 d	46.3 d	53.4 d	52.3 d	56.9 d
6	147.4 s	22.8 t	24.9 t	22.9 t	32.8 t	30.9 t	38.0 t	38.1 t	38.1 t	20.1 t	32.1 t	19.9 t	29.7 t	36.8 t	30.9 t	38.8 t	25.3 t	38.7 t	22.6 t	19.1 t	36.1 t	21.3 t
7	183.1 s	36.4 t	36.0 t	36.7 t	71.9 d	72.1 d	202.6 s	201.2 s	201.2 s	31.3 t	67.9 d	31.1 t	68.2 d	200.2 s	72.3 d	199.5 s	36.4 t	202.5 s	31.1 t	31.6 t	201.5 s	44.8 t
8	153.0 s	169.9 s	168.6 s	169.7 s	166.5 s	166.7 s	154.8 s	154.7 s	154.8 s	137.3 s	138.1 s	136.8 s	137.9 s	132.0 s	167.1 s	132.0 s	169.3 s	154.5 s	135.5 s	135.6 s	131.7 s	73.5 s
9	48.7 d	52.9 d	52.5 d	52.7 d	48.5 d	48.5 d	52.8 d	52.4 d	52.6 d	142.4 s	156.4 s	156.8 s	156.7 s	162.1 s	49.6 d	161.8 s	53.0 d	52.8 d	157.2 s	148.4 s	154.9 s	67.0 d
10	40.5 s	39.9 s	38.9 s	39.9 s	40.2 s	40.6 s	38.9 s	37.1 s	37.1 s	38.3 s	39.2 s	39.4 s	37.6 s	39.6 s	40.7 s	39.5 s	39.5 s	36.8 s	38.9 s	39.4 s	39.9 s	39.2 s
11	23.6 t	21.6 t	21.5 t	21.6 t	21.1 t	21.3 t	24.1 t	24.1 t	24.1 t	126.4 d	125.7 d	125.9 d	126.0 d	126.0 d	21.3 t	125.9 d	21.4 t	23.9 t	125.8 d	125.4 d	125.5 d	147.8 d
12	37.5 t	37.5 t	37.4 t	37.5 t	37.4 t	37.5 t	38.8 t	38.7 t	38.8 t	114.1 d	128.8 d	126.8 d	128.8 d	134.6 d	37.5 t	134.5 d	37.5 t	38.9 t	126.8 d	123.2 d	134.1 d	136.5 d
13	202.1 s	202.8 s	202.1 s	202.7 s	203.2 s	203.4 s	201.6 s	202.6 s	202.6 s	155.6 s	136.1 s	135.6 s	136.0 s	136.4 s	203.4 s	136.6 s	202.6 s	200.8 s	137.0 s	147.6 s	148.2 s	201.1 s
14	130.5 d	126.1 d	126.6 d	126.2 d	128.1 d	128.0 d	130.1 d	130.1 d	130.0 d	115.7 d	132.2 d	130.6 d	132.3 d	128.5 d	128.0 d	128.5 d	126.4 d	130.3 d	130.6 d	126.1 d	125.6 d	
15											200.3 s	200.7 s	200.3 s	199.6 s		199.3 s			200.8 s	72.8 s	34.8 d	
16											26.6 q	26.6 q	26.6 q	26.7 q		26.7 q			26.6 q	31.9 q	24.2 q	27.1 q
17																				31.9 q	24.2 q	24.3 q
18	72.6 t	71.7 t	207.6 d	73.1 t	181.9 s	73.7 t	71.0 t	72.5 t	72.5 t	73.7 t	181.9 s	73.3 t	73.6 t	72.7 t	33.8 q	181.0 s	183.5 s	181.0 s	185.1 s			34.3 q
19	22.7 q	18.4 q	16.1 q	18.3 q	17.5 q	18.0 q	17.6 q	17.5 q	17.5 q	17.8 q	17.1 q	17.9 q	17.8 q	17.6 q	22.4 q	16.9 q	17.9 q	17.0 q	17.8 q	22.8 q	23.0 q	22.0 q
20	27.7 q	16.3 q	15.0 q	16.3 q	15.4 q	15.7 q	15.4 q	15.3 q	15.3 q	25.9 q	24.3 q	25.4 q	24.5 q	23.8 q	15.2 q	23.5 q	15.9 q	15.2 q	25.2 q	24.9 q	22.5 q	16.6 q
1′	174.2 s			174.0 s		174.2 s		174.0 s	173.9 s	174.4 s		174.7 s	174.4 s	175.1 s								
2′	30.3 t			30.3 t		30.4 t		30.2 t	30.0 t	30.3 t		31.1 t	29.9 t	31.6 t								
3′	29.9 t			29.8 t		29.9 t		29.8 t	29.7 t	30.4 t		31.8 t	30.2 t	32.8 t								
4′	175.8 s			175.9 s		175.9 s		175.9 s	174.6 s	176.4 s		177.9 s	176.3 s	179.6 s								
4′-OMe									52.2 q													

[a] Recorded at 75 MHz.
[b] Recorded at 150 MHz.

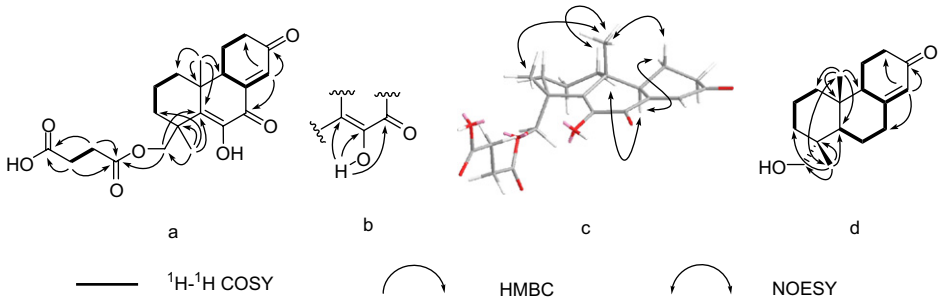

Figure 1. Key ^{1}H–^{1}H COSY, HMBC, and NOESY correlations for compounds **1** and **2**.

Compound **4** was found to possess the molecular formula, $C_{21}H_{30}O_5$, as shown from its positive HRESIMS at *m/z* 385.1968 $[M+Na]^+$. The IR spectrum showed the presence of hydroxyl (3445 cm^{-1}), carbonyl (1739 and 1711 cm^{-1}), and olefinic (1651 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectroscopic data of **4** were very similar to those of **2** except for the additional butanedioic acid moiety [δ_H 2.60 (2H, m, H-2′), 2.61 (2H, m, H-3′); δ_C 29.8 (t, C-3′), 30.3 (t, C-2′), 174.0 (s, C-1′), 175.9 (s, C-4′)]. The downshift of C-18 from δ_C 71.7 to 73.1 as compared with **2** established the connection of the butanedioic acid group to C-18. This assumption was confirmed by the HMBC correlations of H_2-18 with the ester carbonyl of the butanedioic acid at δ_C 175.9. Therefore, compound **4** was concluded to be 18-*O*-butanedioylpodocarpen-8(14)-en-13-one, named abiesanordine D.

Compound **5** was found to possess the molecular formula, $C_{17}H_{24}O_4$, as shown from its negative HRESIMS at *m/z* 291.1594 $[M-H]^-$. Its IR spectrum showed the presence of hydroxyl (3483 cm^{-1}), carbonyl (1724 cm^{-1}), and olefinic (1646 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectroscopic data were similar to those of 8(14)-podocarpen-13-on-18-oic acid (**17**),[6] except for the presence of an additional hydroxyl group at C-7. This was confirmed by the DQF COSY spectrum of the correlations from H-5 through H-6 to H-7, H-14, as well as the long-range correlation of H-14 with C-7 in the HMBC spectrum. Based on the NOESY correlations of Me-19/Me-20 and H-5/H-9 as well as the small coupling constant of H-7 (t, J=3.0 Hz), **5** was then concluded to be 7α-hydroxypodocarpen-8(14)-en-13-on-18-oic acid, named abiesanordine E.

Compound **6** was established the molecular formula, $C_{21}H_{30}O_6$, from the negative HRESIMS at *m/z* 377.1968 $[M-H]^-$. Its UV, IR, and 1D NMR spectroscopic data were very similar to those of **4**. Close comparison of ^{13}C NMR data of these two compounds indicated that compound **6** should have an additional hydroxyl substituent at C-7. By detailed analysis of its 2D NMR spectra, including HSQC, DQF COSY, HMBC, and NOESY, compound **6** was then elucidated as 18-*O*-butanedioylpodocarpen-8(14)-en-7α-hydroxy-13-one, named abiesanordine F.

Compound **7** was assigned the molecular formula $C_{17}H_{24}O_3$ by HRESIMS at *m/z* 299.1605 $[M+Na]^+$. Its NMR spectroscopic data were similar to those of 8(14)-podocarpen-7,13-dion-18-oic acid (**18**),[6] except for the presence of a primary hydroxyl group instead of a carboxyl group of **18**. Accordingly, **7** was determined as 18-hydroxypodocarpen-8(14)-en-7,13-dione, named abiesanordine G.

Compound **8** had the molecular formula $C_{21}H_{28}O_6$ as established from its HRESIMS (*m/z* 399.1716 $[M+Na]^+$). Its ^{13}C NMR spectroscopic data were very similar to those of **7**, except for an additional butanedioic acid moiety [δ_H 2.61 (2H, m, H-2′), 2.60 (2H, m, H-3′); δ_C 30.2 (t, C-2′), 29.8 (t, C-3′), 173.9 (s, C-1′), 175.9 (s, C-4′)]. Thus compound **8** was elucidated as 7α-hydroxypodocarpen-8(14)-en-13-on-18-*O*-butanedioic acid, named abiesanordine H.

Compound **9** had the molecular formula $C_{22}H_{30}O_6$ as established from its HRESIMS (*m/z* 413 $[M+Na]^+$). Its ^{13}C NMR spectroscopic data were very similar to those of **8**, except for an additional methoxyl moiety [δ_H 3.60 (3H, s, 4′-OMe); δ_C 52.2 (q, 4′-OMe)]. Thus compound **9** was elucidated as methyl 7α-hydroxypodocarpen-8(14)-en-13-on-18-*O*-butanedioate, named abiesanordine I.

Compound **10** was assigned the molecular formula $C_{21}H_{28}O_5$ from its negative HRESIMS at *m/z* 359.1841 $[M-H]^-$. The ^{13}C NMR spectroscopic data were similar to those of 17-nor-15-oxo-8,11,13-abietatrien-18-oic acid (**19**)[7] except that a carboxyl group (δ_C 185.1) at C-18 and an acetyl moiety at C-13 in **19** were replaced by an oxygenated methylene (δ_C 73.3) and a hydroxyl, respectively, in **10**. Furthermore, an additional butanedioic acid group was also found in **10** [δ_H 2.54 (2H, m, H-2′), 2.57 (2H, m, H-3′); δ_C 30.3 (t, C-2′), 30.4 (t, C-3′), 174.4 (s, C-1′), 176.4 (s, C-4′)]. According to HMBC correlations of H_2-18 [4.00 (1H, d, J=10.8 Hz, H-18a); 3.71 (1H, d, J=10.8 Hz, H-18b)] to ester carbonyl at δ_C 174.4 (s, C-1′) of butanedioic acid moiety, compound **10** was, therefore, determined to be 13-hydroxypodocarpen-8,11,13-trien-18-*O*-butanedioic acid, named abiesanordine J.

Compound **11** exhibited a $[M+Na]^+$ ion peak at *m/z* 339.1606 in the positive HRESIMS, corresponding to the molecular formula, $C_{19}H_{24}O_4$. The IR spectrum indicated the presence of hydroxyl (3407 cm^{-1}), carbonyl (1728 and 1682 cm^{-1}), and aromatic (1605, 1566, and 1470 cm^{-1}) moieties. The ^{1}H and ^{13}C NMR spectroscopic data of **11** (Tables 1 and 2) showed 19 carbon signals including 2 quaternary and 1 acetyl methyl groups [δ_H 1.16 (s, Me-20), 1.27 (s, Me-19), 2.57 (s, Me-16); δ_C 17.1 (q, Me-19), 24.3 (q, Me-20), 26.6 (q, Me-16), 200.3 (s, C-15)], one ABX system benzene ring [δ_H 7.44 (1H, d, J=8.4 Hz, H-11), 7.85 (1H, dd, J=8.4, 2.1 Hz, H-12), and

7.98 (1H, d, J=2.1 Hz, H-14)]. These signals were very similar to those of 17-nor-15-oxo-8,11,13-abietatrien-18-oic acid (**19**)[7] except for an additional hydroxyl substituent at C-7. This was confirmed by the correlations of H-5/H-6a, H-6a/H-6b, and H-6b/H-7 in the DQF COSY experiment, and H-7 to C-14 in the HMBC spectrum. The 7-OH was determined the same as that in 7α-hydroxypodocarpen-8(14)-en-13-one (**15**) according to the small coupling constant of H-7 (dd, J=4.8, 1.5 Hz). Therefore, compound **11** was assigned as 17-nor-15-oxo-8,11,13-abietatrien-7α-hydroxy-18-oic acid, named abiesanordine K.

Compound **12** was assigned the molecular formula $C_{23}H_{30}O_5$ by HRESIMS at m/z 409.1987 $[M+Na]^+$. The ^1H and ^{13}C NMR spectroscopic data were similar to those of **10** except that the hydroxyl moiety at C-18 in **10** was replaced by an acetyl group in **12**. Therefore, compound **12** was determined to be 17-nor-15-oxo-8,11,13-abietatrien-18-butanedioic acid, named abiesanordine L.

Compound **13** had a molecular formula of $C_{23}H_{30}O_6$ as indicated from its positive HRESIMS at m/z 425.1940 $[M+Na]^+$. Close comparison of the ^{13}C NMR spectrum of compound **13** to those of **12** showed a general similarity except that a methylene in **12** was replaced by an oxygenated methine at δ_C 68.2. Taking the molecular formula into consideration, compound **13** was supposed to be a hydroxyl substituted compound of **12**. Since δ_H 7.96 (1H, d, J=2.1 Hz, H-14) was correlated to δ_C 68.2 in the HMBC spectrum, the hydroxyl was attached to C-7 position. According to the small coupling constant of H-7 (t, J=2.4 Hz), the hydroxyl moiety at C-7 position was established as α-oriented. Therefore, compound **13** was assigned as 17-nor-15-oxo-8,11,13-abietatrien-7α-hydroxy-18-butanedioic acid, named abiesanordine M.

Compound **14** gave a molecular formula $C_{23}H_{28}O_6$ from its HRESIMS at m/z 423.1716 $[M+Na]^+$, and exhibited very similar physical and spectroscopic data to those of **13**. However, inspection of the ^{13}C NMR spectroscopic data of compound **14** established significant differences from those of **13**: an oxygenated methine in **13** was oxidated to be a carboxyl group in **14**. This was coincident with the difference of their molecular formula. Further evidence was found in the HMBC spectrum of H-14 at δ_H 8.51 (1H, d, J=2.1 Hz) to C-7 at δ_C 200.2. Therefore, compound **14** was determined to be 17-nor-7,15-dion-8,11,13-abietatrien-18-butanedioic acid, named abiesanordine N.

All these 22 isolates (**1**–**22**) were tested for inhibitory activities against LPS-induced NO production in RAW264.7 macrophages under the concentration range from 10 to 50 μg/mL. Seven compounds, **2**, **3**, **9**, **14**, **18**, **21**, and **22**, exhibited significant effects with IC_{50} values of 55.7, 35.4, 17.0, 41.7, 60.8, 53.4, and 60.3 μg/mL, respectively (Table 3). These seven bioactive compounds were also tested by MTT assay for cytotoxic activities against RAW264.7 macrophages under the concentration of 50 μg/mL, four compounds, **3**, **14**, **21**, and **22** showed different cytotoxicity with the inhibition rates of 9, 82, 23, and 34%. Instead, the other three compounds, **2**, **9**, and **18** did not show any cytotoxicity at the same concentration.

Abiesanordines A–N (**1**–**14**) and compounds **15**–**22** are the first norditerpenoids reported from the *Abies* species. Although phytochemical investigations were carried out on 19 plants of this genus, studies on *Abies* plants occurring in China have never been reported. Since *A. georgei* is distributed exclusively in China, chemotaxonomic significance of the norditerpenoids as the characteristic of this plant or *Abies* species in China still remains unknown.

Table 3
Inhibitory effects of compounds isolated from *Abies georgei* against LPS-induced NO production in RAW264.7 macrophages and their cytotoxicity (n=4, mean±SD)

Groups	IC_{50}[a] (μg/mL)	Inhibition rate[b] (%)
Aminoguanidine[c]	24.6 (μM)	NT[e]
Abiesanordine B (**2**)	55.7	0
Abiesanordine C (**3**)	35.4	9
Abiesanordine I (**9**)	17.0	0
Abiesanordine N (**14**)	41.7	82
8(14)-Podocarpen-7,13-dion-18-oic acid (**18**)	60.8	0
4-Hydroxy-18-nor-8,11,13-abietatrien-7-one (**21**)	53.4	23
8-Hydroxy-14,15-dinor-11-labden-13-one (**22**)	60.3	34
OCs[d]	>80	NT[e]

[a] Inhibitory effects of compounds **1**–**22** against LPS-induced NO production in RAW264.7 macrophages.
[b] Cytotoxicity effects of compounds **2**, **3**, **9**, **14**, **18**, **21**, and **22** (50 μg/mL) on RAW264.7 macrophages.
[c] Positive control.
[d] Other 15 compounds, including **1**, **4**–**8**, **10**–**13**, **15**–**17**, **19**, and **20**.
[e] Not tested.

3. Experimental

3.1. General

1D and 2D NMR spectra were recorded on a Bruker Avance 600 or Avance 300 NMR spectrometer in CD_3OD with TMS as internal standard. ESIMS and HRESIMS were measured on a Agilent LC/MSD Trap XCT and a Q-TOF micro mass spectrometer (Waters, USA), respectively. Optical rotations were acquired with Perkin–Elmer 341 polarimeter, while CD and UV spectra were obtained using JASCO J810 and Shimadzu UV-2550 UV–visible spectrophotometers, respectively. IR spectra were recorded on a Bruker Vector-22 spectrometer with KBr pellets. Materials for CC were silica gel (100–200, 300–400 mesh, and 10–40 μm; Huiyou Silical Gel Development Co. Ltd., Yantai, PR China), Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-GEL ODS-A (50 μm; YMC, MA, U.S.A.). Preparative TLC (0.4–0.5 mm) was conducted with glass precoated silica gel GF_{254} (Yantai). Compounds were visualized by exposure to UV at 254 nm.

3.2. Plant material

The aerial parts of *A. georgei* 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 was deposited in School of Pharmacy, Second Military Medical University, China (herbarium No. 2006–07-016).

3.3. 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 and concentrated 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 (100–200 mesh) eluting with gradient $CHCl_3$/Me_2CO. Fraction F_1 (36.3 g) was subjected to column chromatography (CC) over MCI and Sephadex LH-20 to give **3** (1.2 mg) and **14** (3.4 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 **7** (8.6 mg), **16** (6.4 mg), and **18** (30.0 mg) were obtained from subfraction $F_{2\text{-}4}$ (976.4 mg) after CC over Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) followed by repeated preparative TLC using $CHCl_3$/MeOH (20:1). By the same procedures, **15** (1.1 mg) and **17** (5.1 mg) were obtained from subfraction $F_{2\text{-}5}$ (712.1 mg), **12** (2.7 mg) and **21** (3.4 mg) from subfraction $F_{2\text{-}8}$; **2** (3.4 mg), **19** (12.7 mg), **20** (3.9 mg), and **22** (28.5 mg) from $F_{2\text{-}6}$, $F_{2\text{-}7}$, $F_{2\text{-}19}$, and $F_{2\text{-}9}$, respectively. Fraction F_3 (27.0 g) was subjected to CC over ODS [MeOH/H_2O (5:95–100:0)] and Sephadex LH-20 ($CHCl_3$/MeOH, 1:1; MeOH), followed by preparative TLC using $CHCl_3$/MeOH (20:1) and/or petroleum ether/EtOAc (1:1) to give **1** (4.0 mg), **4** (15.8 mg), **9** (2.3 mg), and **14** (10.8 mg). Similarly, **5** (30.7 mg), **6** (7.3 mg), **11** (12.2 mg), and **13** (11.4 mg) were isolated from fraction F_4 after CC over ODS [MeOH/H_2O (5:95–100:0)] and Sephadex LH-20 ($CHCl_3$/MeOH, 1:1; MeOH), followed by preparative TLC using $CHCl_3$/MeOH (20:1) and/or petroleum ether/EtOAc (1:1).

3.4. Biological assays

3.4.1. Inhibitory activities against LPS-stimulated NO production in RAW264.7 macrophages

RAW264.7 macrophages were seeded in 24-well plates (10^5 cells/well). The cells were co-incubated with drugs and LPS (1 μg/mL) for 24 h. The amount of NO was assessed by determining the nitrite concentration in the cultured RAW264.7 macrophage supernatants with Griess reagent. Aliquots of supernatants (100 μL) were incubated, in sequence, with 50 μL 1% sulphanilamide and 50 μL 0.1% naphthylethylenediamine in 2.5% phosphoric acid solution. The absorbances at 570 nm were read using a microtiter plate reader.

3.4.2. MTT assay for cytotoxic activity in RAW264.7 macrophages

RAW264.7 macrophages were maintained in a water-saturated atmosphere of 5% CO_2 at 37 °C. Experiments were carried out according to the reported protocol. The cell viability was evaluated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma] reduction.

*3.4.2.1. Abiesanordine A (**1**).* Amorphous powder; $[\alpha]_D^{20}$ −14.0 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.10), 232 (3.77), 263 (3.70), 347 (3.46); CD (MeOH) $\Delta\varepsilon_{235}$ +29.6, $\Delta\varepsilon_{331}$ −16.0; IR (KBr) ν_{max} 3384, 2963, 2926, 2854, 1738, 1646, 1588, 1466, 1384, 1273, 1153 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 413 $[M+Na]^+$, 803 $[2M+Na]^+$; ESIMS (negative) 425 $[M+Cl]^-$, 779 $[2M-H]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 413.1567, calcd for $C_{21}H_{26}O_7Na$ 413.1576.

*3.4.2.2. Abiesanordine B (**2**).* Amorphous powder; $[\alpha]_D^{20}$ −0.8 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε): 242 (4.00); CD (MeOH) $\Delta\varepsilon_{215}$ +32.8, $\Delta\varepsilon_{242}$ +51.8, $\Delta\varepsilon_{252}$ −13.8; IR (KBr) ν_{max} 3433, 2956, 2926, 2868, 1726, 1660, 1446, 1392, 1265, 1218, 1061, 876 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 285 $[M+Na]^+$, 547 $[2M+Na]^+$; HRESIMS (positive) $[M+Na]^+$ *m/z* 285.1811, calcd for $C_{17}H_{26}O_2Na$ 285.1831.

*3.4.2.3. Abiesanordine C (**3**).* Amorphous powder; $[\alpha]_D^{20}$ −2.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 209 (3.68), 237 (3.42); CD (MeOH) $\Delta\varepsilon_{223}$ +6.4, $\Delta\varepsilon_{244}$ +8.8, $\Delta\varepsilon_{316}$ −2.6; IR (KBr) ν_{max} 2960, 2928, 2851, 1724, 1709, 1662, 1465, 1260, 1044 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 283 $[M+Na]^+$, 543 $[2M+Na]^+$; HRESIMS (positive) $[M+H]^+$ *m/z* 261.1860, calcd for $C_{17}H_{25}O_2$ 261.1855.

*3.4.2.4. Abiesanordine D (**4**).* Amorphous powder; $[\alpha]_D^{20}$ −14.3 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε): 211 (4.00), 240 (3.89), 281 (3.58); CD (MeOH) $\Delta\varepsilon_{223}$ +23.6, $\Delta\varepsilon_{243}$ +28.3, $\Delta\varepsilon_{312}$ −7.6; IR (KBr) ν_{max} 3445, 2928, 2851, 1739, 1711, 1651, 1472, 1384, 1263, 1160, 998, 877 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 385 $[M+Na]^+$, 747 $[2M+Na]^+$; ESIMS (negative) *m/z* 361 $[M-H]^-$, 723 $[2M-H]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 385.1968, calcd for $C_{21}H_{30}O_5Na$ 385.1991.

*3.4.2.5. Abiesanordine E (**5**).* Amorphous powder; $[\alpha]_D^{20}$ −79.0 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε): 214 (3.86), 237 (4.12); CD (MeOH) $\Delta\varepsilon_{234}$ +16.5, $\Delta\varepsilon_{326}$ −19.3; IR (KBr) ν_{max} 3483, 2948, 2864, 1724, 1646, 1454, 1385, 1262, 1185, 1140, 1041, 877 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 315 $[M+Na]^+$, 607 $[2M+Na]^+$; ESIMS (negative) *m/z* 291 $[M-H]^-$, 583 $[2M-H]^-$; HRESIMS (negative) $[M-H]^-$ *m/z* 291.1594, calcd for $C_{17}H_{23}O_4$ 291.1596.

*3.4.2.6. Abiesanordine F (**6**).* Amorphous powder; $[\alpha]_D^{20}$ −30.0 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε): 212 (3.82), 247 (4.21); CD (MeOH) $\Delta\varepsilon_{243}$ +9.4, $\Delta\varepsilon_{325}$ −9.8; IR (KBr) ν_{max} 3439, 2929, 1737, 1711, 1680, 1395, 1262, 1038, 952, 879 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 401 $[M+Na]^+$; HRESIMS (negative) $[M-H]^-$ *m/z* 377.1968, calcd for $C_{21}H_{29}O_6$ 377.1964.

*3.4.2.7. Abiesanordine G (**7**).* Amorphous powder; $[\alpha]_D^{20}$ −46.2 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε): 213 (3.80), 255 (3.63), 318 (3.10); CD (MeOH) $\Delta\varepsilon_{245}$ +4.1, $\Delta\varepsilon_{332}$ −4.7; IR (KBr) ν_{max} 3433, 2928, 2870, 1779, 1725, 1675, 1467, 1392, 1225, 1195, 1115, 1031, 976, 793 cm^{-1}; for ^1H and ^{13}C NMR

data, see Tables 1 and 2; ESIMS (positive) *m/z* 277 $[M+H]^+$, 299 $[M+Na]^+$, 575 $[2M+Na]^+$; HRESIMS (positive) $[M+Na]^+$ *m/z* 299.1605, calcd for $C_{17}H_{24}O_3Na$ 299.1623.

*3.4.2.8. Abiesanordine H (**8**).* Amorphous powder; $[\alpha]_D^{20}$ −33.3 (*c* 0.41; MeOH); UV (MeOH) λ_{max} (log ε): 215 (3.78), 234 (3.78), 252 (3.80); CD (MeOH) $\Delta\varepsilon_{234}$ +26.1, $\Delta\varepsilon_{270}$ −7.4; IR (KBr) ν_{max} 3420, 2923, 2852, 1738, 1678, 1471, 1384, 1154, 999, 829 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 377 $[M+H]^+$, 399 $[M+Na]^+$, 775 $[M+Na]^+$; ESIMS (negative) *m/z* 375 $[M-H]^-$, 411 $[M+Cl]^-$, 751 $[2M-H]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 399.1716, calcd for $C_{21}H_{28}O_6Na$ 399.1784.

*3.4.2.9. Abiesanordine I (**9**).* Amorphous powder; $[\alpha]_D^{20}$ −4.7 (*c* 0.33; MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.03), 232 (4.02), 254 (3.98); CD (MeOH) $\Delta\varepsilon_{234}$ +35.5, $\Delta\varepsilon_{270}$ −12.0; IR (KBr) ν_{max} 3429, 2928, 2866, 1771, 1725, 1679, 1552, 1469, 1367, 1222, 1188, 1028, 962, 810 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 413 $[M+Na]^+$; HRESIMS (positive) $[M+Na]^+$ *m/z* 413.1912, calcd for $C_{22}H_{30}O_6Na$ 413.1940.

*3.4.2.10. Abiesanordine J (**10**).* Amorphous powder; $[\alpha]_D^{20}$ +33.5 (*c* 0.34; MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.23), 255 (3.98), 282 (3.51); CD (MeOH) $\Delta\varepsilon_{228}$ +24.7, $\Delta\varepsilon_{257}$ −6.6, $\Delta\varepsilon_{342}$ +6.4; IR (KBr) ν_{max} 3423, 2963, 2926, 2852, 1740, 1709, 1608, 1518, 1383, 1262, 1104, 1028, 801 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 383 $[M+Na]^+$; ESIMS (negative) *m/z* 359 $[M-H]^-$, 719 $[2M-H]^-$; HRESIMS (negative) $[M-H]^-$ *m/z* 359.1841, calcd for $C_{21}H_{27}O_5$ 359.1858.

*3.4.2.11. Abiesanordine K (**11**).* Amorphous powder; $[\alpha]_D^{20}$ +2.1 (*c* 0.50; MeOH); UV (MeOH) λ_{max} (log ε): 211 (3.86), 237 (4.12); CD (MeOH) $\Delta\varepsilon_{222}$ +5.7, $\Delta\varepsilon_{247}$ +16.8, $\Delta\varepsilon_{288}$ −3.5, $\Delta\varepsilon_{323}$ +2.5; IR (KBr) ν_{max} 3407, 2928, 2867, 2630, 1728, 1682, 1605, 1566, 1470, 1360, 1282, 1253, 1188, 1048, 953, 835, 720 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 339 $[M+Na]^+$, 655 $[2M+Na]^+$; HRESIMS (positive) $[M+Na]^+$ *m/z* 339.1606, calcd for $C_{19}H_{24}O_4Na$ 339.1572.

*3.4.2.12. Abiesanordine L (**12**).* Amorphous powder; $[\alpha]_D^{20}$ +1.8 (*c* 0.42; MeOH); UV (MeOH) λ_{max} (log ε): 215 (4.24), 257 (4.05), 282 (3.28); CD (MeOH) $\Delta\varepsilon_{235}$ +2.0, $\Delta\varepsilon_{255}$ +13.8; IR (KBr) ν_{max} 3428, 2927, 2868, 1736, 1680, 1603, 1564, 1416, 1358, 1268, 1161, 998, 830, 669 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 409 $[M+Na]^+$, 795 $[2M+Na]^+$; ESIMS (negative) *m/z* 385 $[M-H]^-$, 771 $[2M-H]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 409.1987, calcd for $C_{23}H_{30}O_5Na$, 409.1991.

*3.4.2.13. Abiesanordine M (**13**).* Amorphous powder; $[\alpha]_D^{20}$ −3.2 (*c* 0.50; MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.10), 254 (3.87); CD (MeOH) $\Delta\varepsilon_{243}$ +13.5, $\Delta\varepsilon_{252}$ −7.2, $\Delta\varepsilon_{262}$ +3.4; IR (KBr) ν_{max} 3446, 2930, 2853, 1737, 1710, 1682, 1604, 1471, 1373, 1280, 1160, 1053, 832 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 425 $[M+Na]^+$, 827 $[2M+Na]^+$; ESIMS (negative) *m/z* 402 $[M-H]^-$, 803 $[2M-H]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 425.1933, calcd for $C_{23}H_{30}O_6Na$ 425.1940; HRESIMS (negative) $[M-H]^-$ *m/z* 401.1962, calcd for $C_{23}H_{29}O_6$ 401.1964.

*3.4.2.14. Abiesanordine N (**14**).* Amorphous powder; $[\alpha]_D^{20}$ +123.6 (*c* 0.50; MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.23), 233 (4.26), 255 (3.91), 292 (3.11); CD (MeOH) $\Delta\varepsilon_{224}$ +15.5, $\Delta\varepsilon_{256}$ −20.1, $\Delta\varepsilon_{324}$ +9.5; IR (KBr) ν_{max} 3433, 2929, 2849, 1740, 1643, 1604, 1467, 1410, 1350, 1164, 956, 919, 889 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 423 $[M+Na]^+$; ESIMS (negative) *m/z* 399 $[M-H]^-$, 799 $[2M-H]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 423.1716, calcd for $C_{23}H_{28}O_6Na$ 423.1784.

*3.4.2.15. 7α-Hydroxypodocarpen-8(14)-en-13-one (**15**).* Amorphous powder; $[\alpha]_D^{20}$ −8.5 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε): 212 (3.73), 236 (3.60); CD (MeOH) $\Delta\varepsilon_{243}$ +3.9, $\Delta\varepsilon_{326}$ −6.0; IR (KBr) ν_{max} 3473, 2960, 2922, 2868, 1687, 1626, 1466, 1394, 1258, 1110, 774 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 285 $[M+Na]^+$, 547 $[2M+Na]^+$; HRESIMS (positive) $[M+Na]^+$ *m/z* 285.1814, calcd for $C_{17}H_{26}O_2Na$ 285.1831.

*3.4.2.16. 17-Nor-7,15-dion-8,11,13-abietatrien-18-oic acid (**16**).* Amorphous powder; $[\alpha]_D^{20}$ +12.0 (*c* 0.50; MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.02), 232 (4.41), 254 (4.06), 285 (3.35); CD (MeOH) $\Delta\varepsilon_{230}$ +22.4, $\Delta\varepsilon_{257}$ −27.8, $\Delta\varepsilon_{324}$ +12.4; IR (KBr) ν_{max} 3365, 2933, 2870, 1727, 1690, 1600, 1513, 1471, 1408, 1360, 1236, 1074, 839 cm^{-1}; for ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive) *m/z* 315 $[M+H]^+$, 337 $[M+Na]^+$, 651 $[2M+Na]^+$; ESIMS (negative) *m/z* 313 $[M-H]^-$, 627 $[2M-H]^-$; HRESIMS (positive) $[M+Na]^+$ *m/z* 337.1451, calcd for $C_{19}H_{22}O_4Na$ 337.1416.

*3.4.2.17. 8(14)-Podocarpen-13-on-18-oic acid (**17**).* Amorphous powder; ^1H NMR (CD_3OD, 300 MHz) δ 0.86 (3H, s, Me-20), 1.20 (3H, s, Me-19), 2.15 (1H, dd, *J*=12.3, 2.7 Hz, H-5), 2.50 (1H, dd, *J*=10.5, 3.6 Hz, H-7a), 5.85 (1H, br s, H-14); ^{13}C NMR, see Table 2; ESIMS (positive) *m/z* 299 $[M+Na]^+$, 575 $[2M+Na]^+$; ESIMS (negative) *m/z* 275 $[M-H]^-$, 551 $[2M-H]^-$.

*3.4.2.18. 8(14)-Podocarpen-7,13-dion-18-oic acid (**18**).* Amorphous powder; ^1H NMR (CD_3OD, 300 MHz) δ 0.83 (3H, s, Me-20), 1.27 (3H, s, Me-19), 2.61 (1H, dt, *J*=10.5, 3.6 Hz, H-9), 6.51 (1H, dd, *J*=3.0, 1.2 Hz, H-14); ^{13}C NMR, see Table 2; ESIMS (positive) *m/z* 291 $[M+H]^+$, 313 $[M+Na]^+$, 603 $[2M+Na]^+$; ESIMS (negative) *m/z* 289 $[M-H]^-$, 325 $[M+Cl]^-$, 579 $[2M-H]^-$.

*3.4.2.19. 17-Nor-15-oxo-8,11,13-abietatrien-18-oic acid (**19**).* Amorphous powder; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.21 (3H, s, Me-20), 1.24 (3H, s, Me-19), 2.54 (3H, s, Me-16), 2.22 (1H, dd, J=12.6, 2.2 Hz, H-5), 2.36 (1H, br d, J=12.3 Hz, H-1a), 2.94 (2H, m), 7.12 (1H, dd, J=8.1, 1.8 Hz, H-12), 7.38 (1H, d, J=8.1 Hz, H-11), 7.65 (1H, d, J=1.8 Hz, H-14); ^{13}C NMR, see Table 2; ESIMS (positive) *m/z* 323 $[M+Na]^+$, 623 $[2M+Na]^+$; ESIMS (negative) *m/z* 299 $[M-H]^-$, 599 $[2M-H]^-$.

*3.4.2.20. 18-Nor-abieta-8,11,13-triene-4,15-diol (**20**).* Amorphous powder; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.13 (3H, s, Me-20), 1.19 (3H, s, Me-19), 1.48 (6H, s, Me-16, Me-17), 2.13 (1H, dd, J=12.9, 6.9 Hz, H-5), 2.28 (1H, br d, J=12.6 Hz, H-1a), 2.89 (2H, m, H-7), 7.12 (1H, s, H-14), 7.18 (2H, br s, H-11,H-12); ^{13}C NMR, see Table 2; ESIMS (positive) *m/z* 311 $[M+Na]^+$, 599 $[2M+Na]^+$.

*3.4.2.21. 4-Hydroxy-18-nor-8,11,13-abietatrien-7-one (**21**).* Amorphous powder; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.23 (6H, d, J=6.9 Hz, Me-16,17), 1.25 (3H, s, Me-19), 1.27 (3H, s, Me-20), 2.10 (1H, dd, J=14.1, 3.9 Hz, H-5), 2.67 (1H, dd, J=14.1, 3.0 Hz, H-6a), 2.89 (1H, d, J=14.1 Hz, H-6b), 2.93 (1H, m, H-15), 7.40 (1H, d, J=8.1 Hz, H-11), 7.48 (1H, dd, J=8.1, 2.1 Hz, H-12), 7.82 (1H, d, J=2.1 Hz, H-14); ^{13}C NMR, see Table 2; ESIMS (positive) *m/z* 309 $[M+Na]^+$, 595 $[2M+Na]^+$.

*3.4.2.22. 8-Hydroxy-14,15-dinor-11-labden-13-one (**22**).* Amorphous powder; ^{1}H NMR (CD_3OD, 300 MHz) δ 0.84 (3H, s, Me-20), 0.89 (3H, s, Me-19), 0.96 (1H, dd, J=11.7, 2.1 Hz, H-5),1.02 (3H, s, Me-18), 1.25 (3H, s, Me-17), 2.28 (3H, s, Me-16), 1.88 (1H, dt, J=12.3, 3.0 Hz, H-6a), 1.98 (1H, d, J=10.5 Hz, H-9), 6.12 (1H, d, J=15.6 Hz, H-12), 6.93 (1H, dd, J=15.6, 10.5 Hz, H-11); ^{13}C NMR, see Table 2; ESIMS (positive) *m/z* 301 $[M+Na]^+$, 579 $[2M+Na]^+$.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.02.069.

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