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Stelleralides D−J and Anti-HIV Daphnane Diterpenes from *Stellera chamaejasme*

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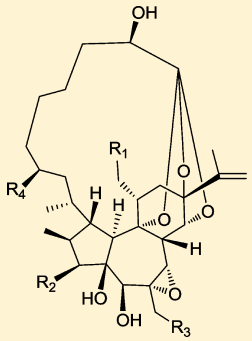
Supporting Information



compound	anti-HIV EC_{50} (μM)	cytotoxicity CC_{50} (μM)	selectivity index CC_{50} / EC_{50}
3	0.00093	>12	>12900
4	0.00073	>11.2	>15300
5	0.00098	>12.9	>13100

	R_1	R_2	R_3	R_4
3	OAc	OBz	OH	OBz
4	OBz	OBz	OH	OBz
5	OBz	OH	OBz	H

ABSTRACT: Bioassay-guided fractionation of a petroleum ether extract of the roots of *Stellera chamaejasme* led to the isolation of seven new (stelleralides D−J, **1**−**7**) and 12 known (**8**−**19**) daphnane diterpenoids. The structures and relative configurations of **1**−**7** were established on the basis of extensive spectroscopic analysis, including HRESIMS and comprehensive NMR techniques. All isolates were evaluated for anti-HIV activity in MT4 cells. All compounds tested, except **2**, showed anti-HIV activity, and, especially, five 1α-alkyldaphnane diterpenoids (**3**, **4**, **5**, **10**, and **11**) exhibited extremely potent anti-HIV activity, with EC_{50} values of 0.06−1.1 nM and selectivity index values of more than 10 000.

The rapid, worldwide spread of acquired immunodeficiency syndrome (AIDS) has prompted an intense research effort to discover compounds that can inhibit effectively the human immunodeficiency virus (HIV).[1,2] Indeed, highly active antiretroviral therapy (HAART), which combines three to four antiretrovirals, can effectively control plasma viremia in many patients, although the virus is suppressed rather than truly eradicated.[3−5] Thus, a major goal of current AIDS therapy continues to be the development of new anti-HIV compounds as well as drug regimens for eradication of the AIDS virus.

Stellera chamaejasme L. (Thymelaeaceae), a toxic perennial herb, is distributed prevalently in northern and southwestern mainland China and Nepal. Its dried roots, named "Rui-Xiang-Lang-Du" in traditional Chinese medicine, have long been used for the treatment of stubborn skin ulcers, tinea, scabies, tuberculosis, and chronic tracheitis.[6] Previous chemical investigations on the roots of this plant have led to the isolation of diverse secondary metabolites, including highly functionalized daphnane diterpenoids,[7−9] tigliane diterpenoids,[10] lignans,[11,12] biflavonoids,[13−16] coumarins,[17,18] and sesquiterpenoids.[11,19] In particular, certain daphnane and tigliane diterpenoids have shown extremely potent anti-HIV activity at low nanomolar concentrations with relatively low cytotoxicity.[7,10] However, these compounds are very difficult to synthesize because of their complex structure. Accordingly, further analysis of the plant material was conducted to acquire a significant quantity of several known, as well as new, anti-HIV diterpenoids from *S. chamaejasme*.

Consequently, the current research on anti-HIV diterpenoids from *S. chamaejasme* led to the isolation of 19 daphnane diterpenoids, including seven new [stelleralides D−J (**1**−**7**)] and 12 known compounds [stelleralide C (**8**),[7] pimelotide A (**9**),[20] gnidimacrin (**10**),[7] pimelea factor P2 (**11**),[7] wikstroelide F (**12**),[7] wikstroelide A (**13**),[21] wikstroelide B (**14**),[22] wikstrotoxin A (**15**),[23] huratoxin (**16**),[7] wikstroelide M (**17**),[22] wikstroelide J (**18**),[22] and simplexin (**19**)[7]]. Herein, are described the isolation and structural elucidation of the new compounds together with the anti-HIV activity of all daphnane diterpenoids isolated.

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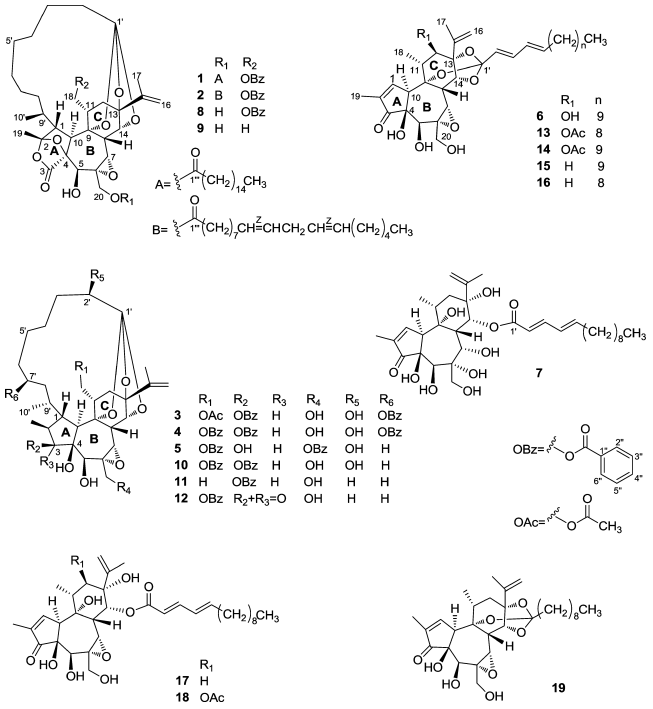

RESULTS AND DISCUSSION

In a bioassay-guided fractionation of an ethanolic extract (EC_{50} = 0.42 ± 0.08 μg/mL, selectivity index (SI) ≥ 23) of the roots of *S. chamaejasme*, three solvent fractions (petroleum ether, EtOAc, *n*-BuOH) were obtained. The petroleum ether-soluble fraction exhibited promising anti-HIV activity with an EC_{50} value of 0.06 ± 0.007 μg/mL (SI ≥ 320), while the EtOAc-soluble fraction had a higher EC_{50} value of 1.26 ± 0.19 μg/mL (SI ≥ 15), and the *n*-BuOH-soluble fraction showed no anti-HIV activity. The active fraction was separated by repeated column chromatography and preparative HPLC to afford 19 daphnane diterpenoids.

Compound **1** was obtained as a colorless oil. Its molecular formula was determined as $C_{53}H_{76}O_{12}$ based on an HRESIMS ion at *m/z* 927.5235 $[M + Na]^+$ (calcd for $C_{53}H_{76}O_{12}Na$, 927.5229), indicating a hydrogen deficiency index (HDI) of 16. The IR spectrum revealed the presence of hydroxy (3478 cm^{-1}), carbonyl (1799 and 1718 cm^{-1}), and aromatic groups (1648 and 1452 cm^{-1}). The ^1H NMR data (Table 1) of **1** indicated two tertiary methyl (δ_H 1.73 and 1.79, each 3H, s), one secondary methyl (δ_H 1.02, 3H, d, *J* = 6.4 Hz), one primary methyl (δ_H 0.86, 3H, t, *J* = 8.0 Hz), two oxygenated methylenes (δ_H 4.09, 4.54, 4.68, and 4.97), three oxygenated methines (δ_H 3.44, 4.35, and 4.82), a terminal double bond (δ_H 5.05 and 4.94), and five aromatic [δ_H 7.57 (1H, dd, *J* = 8.4, 1.2 Hz), 7.46 (2H, dd, *J* = 8.0, 8.0 Hz), 8.04 (2H, dd, *J* = 8.4, 1.2 Hz)] protons. Analysis of the ^{13}C NMR and DEPT spectra of **1** established the presence of daphnane ketal-lactone skeleton resonances[20] including a characteristic quaternary carbon resonance at δ_C 120.4 (C-1′), an acetal carbon resonance at δ_C 112.1 (C-2), a lactone carbonyl carbon resonance at δ_C 173.9 (C-3), and a methyl carbon resonance at δ_C 19.6 (C-19). Comparison of the ^1H and ^{13}C NMR data (Table 1) of **1** with those of stelleralide C (**8**)[7] implied the similarity of essential structural signals. Additionally, the presence of a fatty acid was suggested [δ_C 173.8 (C-1‴), 34.1 (C-2‴), 24.7 (C-3‴), 29.0–29.8 (C-4‴–C-13‴), 31.9 (C-14‴), 22.7 (C-15‴), 14.1 (C-16‴)]. On the basis of the molecular formula, the fatty acid was

Table 1. ^1H (600 MHz) and ^{13}C (150 MHz) NMR Spectroscopic Data for Compounds 1 and 2 in $CDCl_3$

	1[a]		**2**[b]	
position	δ_H [*J* in (Hz)]	δ_C	δ_H [*J* in (Hz)]	δ_C
1	2.86 (1H, dd, 14.0, 4.0)	54.5	2.85 (1H, dd, 12.0, 4.0)	54.5
2		112.1		112.1
3		173.9		173.9
4		86.5		86.5
5	4.82 (1H, brs)	69.3	4.82 (1H, brs)	69.3
6		57.1		57.1
7	3.44 (1H, brs)	59.3	3.43 (1H, brs)	59.3
8	3.26 (1H, dd, 2.8, 1.6)	34.8	3.26 (1H, dd, 2.8, 1.6)	34.8
9		80.1		80.1
10	2.89 (1H, d, 4.0)	54.8	2.89 (1H, d, 4.2)	54.8
11	2.19 (1H, t, 6.8, 6.8)	42.8	2.19 (1H, t, 6.8, 7.2)	42.8
12	2.08 (1H, dd, 11.2, 6.8)	32.6	2.08 (1H, dd, 11.2, 6.8)	32.6
	2.32 (1H, dd, 11.2, 6.8)		2.32 (1H, dd, 11.2, 6.8)	
13		83.4		83.4
14	4.35 (1H, s)	80.6	4.35 (1H, s)	80.6
15		145.7		145.7
16	4.94 (1H, dd, 1.8, 1.8)	111.6	4.94 (1H, dd, 2.0, 2.0)	111.6
	5.05 (1H, s)		5.03 (1H, s)	
17	1.79 (3H, s)	18.8	1.79 (3H, s)	18.7
18	4.54 (1H, dd, 12.4, 6.8)	69.5	4.54 (1H, dd, 12.4, 6.8)	69.5
	4.97 (1H, d, 12.5)		4.97 (1H, d, 12.5)	
19	1.73 (3H, s)	19.6	1.74 (3H, s)	19.5
20	4.09, 4.68 (2H, d, 12.5)	63.9	4.09, 4.68 (2H, d, 12.5)	63.9
1′		120.4		120.4
2′	2.10 (1H, ddd, 12.0, 6.0, 3.0)	31.3	2.10 (1H, ddd, 12.0, 6.0, 3.0)	31.3
	1.93 (1H, ddd, 12.0, 5.6, 3.0)		1.93 (1H, ddd, 12.0, 5.6, 3.0)	
3′	1.56, 1.76 (2H, m)	22.2	1.56, 1.76 (2H, m)	22.1
4′	1.27, 1.62 (2H, m)	25.1	1.27, 1.62 (2H, m)	25.1
5′	1.17, 1.48 (2H, m)	24.2	1.17, 1.48 (2H, m)	24.2
6′	1.27, 1.52 (2H, m)	26.6	1.27, 1.52 (2H, m)	26.6
7′	1.11, 1.52 (2H, m)	26.9	1.11, 1.52 (2H, m)	26.9
8′	0.87, 2.27 (2H, m)	32.6	0.87, 2.31 (2H, m)	32.6
9′	1.13 (1H, m)	37.8	1.13 (1H, m)	37.8
10′	1.02 (3H, d, 6.4)	19.2	1.02 (3H,d, 6.4)	19.2
Bz-CO		166.4		166.4
Bz-1″		130.0		130.0
Bz-2″, 6″	8.04 (2H, dd, 8.4, 1.2)	129.5	8.04 (2H, dd, 11.2, 1.2)	129.5
Bz-3″, 5″	7.46 (2H, dd, 8.0, 8.0)	128.5	7.45 (2H, dd, 7.6, 7.6)	128.5
Bz-4″	7.57 (1H, dd, 7.2, 1.2)	133.2	7.57 (1H, dd, 7.6, 1.2)	133.2

[a]Data for palmitic acid group: δ_H 2.37 (2H, t, 8.0, H-2‴), 1.62 (2H, m, H-3‴), 1.23–1.30 (24H, m, H-4‴–H-15‴), 0.86 (3H, t, 8.0, H-16‴); δ_C 173.8 (C-1‴), 34.1 (C-2‴), 24.7 (C-3‴), 29.0–29.8 (C-4‴–C-13‴), 31.9 (C-14‴), 22.7 (C-15‴), 14.1 (C-16‴). [b]Data for linoleic acid group: δ_H 2.38 (2H, t, 8.0, H-2‴), 1.62 (2H, m, H-3‴), 1.23–1.30 (12H, m, H-4‴–H-7‴, H-16‴, and H-17‴), 2.04 (4H, m, H-8‴, H-14‴), 5.32 (1H, m, H-9‴), 5.37 (1H, m, H-10‴), 2.76 (4H, t, 5.2, H-11‴, H-15‴), 5.35 (1H, m, H-12‴), 5.33 (1H, m, H-13‴), and 0.88 (3H, t, 6.8, H-18‴); δ_C 174.0 (C-1‴), 33.9 (C-2‴), 24.7 (C-3‴), 29.0–29.8 (C-4‴–C-7‴), 27.1 (C-8‴, C-14‴), 127.9 (C-9‴), 130.2 (C-10‴), 25.6 (C-11‴, C-15‴), 130.1 (C-12‴), 128.1 (C-13‴), 31.7 (C-16‴), 22.5 (C-17‴), and 14.0 (C-18‴).

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proposed as palmitic acid. The linkage of palmitic acid at C-20 of the diterpenoid skeleton was confirmed by the HMBC correlation between H_2-20 (δ_H 4.09 and 4.68) and the carbonyl carbon (δ_C 173.8) of palmitic acid (Figure 1) as well as the downfield chemical shift of C-20 from δ_C 63.0 in **8** to δ_C 63.9 in **1**. Other correlations in the HMBC spectrum confirmed the connectivities in this compound.

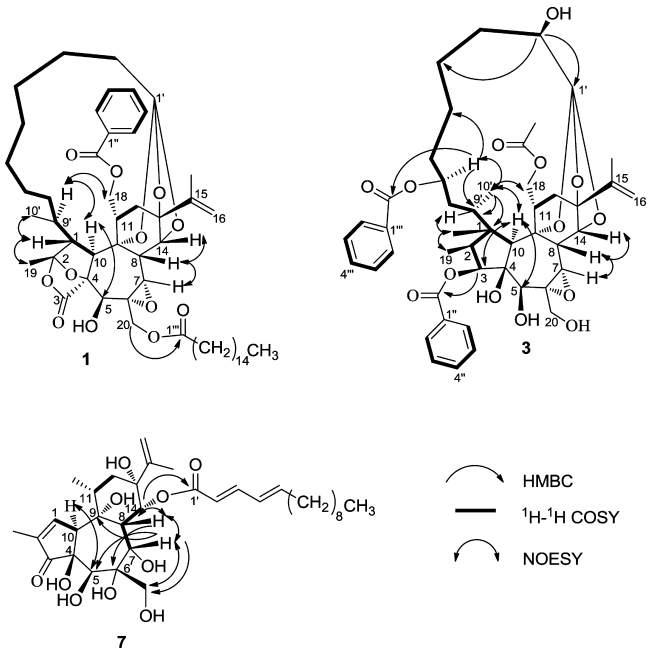

Figure 1. Key ^{1}H−^{1}H COSY, HMBC, and NOESY correlations of **1**, **3**, and **7**.

The relative configuration of **1** was mainly established by comparison of its spectroscopic data with those of stelleralide C (**8**), as well as NOESY results (Figure 1) and molecular modeling calculations. The NOESY correlations of H-1/H-19, H-10′/H-1, H-7/H-8, and H-8/H-14 indicated that these protons are cofacial and β-oriented, while the correlations of H-10/H-9′, H-9′/H_2-18, and H-5/H-10 suggested that these protons are α-oriented. Thus, the structure of **1** (stelleralide D) was established as shown.

The molecular formula of compound **2**, obtained as a colorless oil, was assigned as $C_{55}H_{76}O_{12}$ according to its HRESIMS (m/z 951.5184 $[M + Na]^+$, calcd for $C_{55}H_{76}O_{12}Na$, 951.5229), with an HDI of 18. The IR spectrum indicated the presence of hydroxy (3459 cm^{-1}), carbonyl (1797 and 1717 cm^{-1}), and aromatic (1455 cm^{-1}) groups. On the basis of the ^{1}H and ^{13}C NMR spectra (Table 1), it was evident that **2** has the same daphnane ketal-lactone skeleton as **1**. The key difference between the two compounds was in the lipid motif. The signals due to olefin protons (δ_H 5.32, 5.33, 5.35, and 5.37) were consistent with the presence of an unsaturated fatty acid. The lipid group was defined as linoleic acid by analysis of the appropriate carbon signals [δ_C 174.0 (C-1‴), 33.9 (C-2‴), 24.7 (C-3‴), 29.0−29.8 (C-4‴−C-7‴), 27.1 (C-8‴, C-14‴), 127.9 (C-9‴), 130.2 (C-10‴), 25.6 (C-11‴, C-15‴), 130.1 (C-12‴), 128.1 (C-13‴), 31.7 (C-16‴), 22.5 (C-17‴), and 14.0 (C-18‴)]. In order to confirm the structure of the linoleic acid moiety, a GC-MS experiment[24] was conducted (see Experimental Section). The attachment of the linoleic acid group to C-20 was assigned by the key HMBC correlations from H_2-20 (δ_H 4.09 and 4.68) to C-1‴ (δ_C 174.0). The relative configurations of **2** were determined to be the same as those of **1** by analysis of its NOESY spectrum and molecular modeling, with β-orientations of H-1, H-7, H-8, H-14, H_3-19, and H-10′ and α-orientations of H-5, H-10, H_2-18, and H-9′. The structure of **2** (stelleralide E) was thus established as shown.

Compound **3** was isolated as a white, amorphous powder. Its HRESIMS exhibited a pseudomolecular peak at m/z 855.3557 $[M + Na]^+$ (calcd for $C_{46}H_{56}O_{14}Na$, 855.3562), corresponding to the molecular formula $C_{46}H_{56}O_{14}$. In accordance with the molecular formula, 46 carbon signals were evident in the ^{13}C NMR spectrum. The ^{1}H and ^{13}C NMR spectra of **3** indicated the presence of four methyl groups [δ_C 18.9, δ_H 1.80 (3H, s); δ_C14.5, δ_H 1.18 (3H, d, J = 6.6 Hz); δ_C 18.5, δ_H 1.22 (3H, d, J = 7.2 Hz); δ_C 21.1, δ_H 2.10 (3H, s)], a terminal double bond [δ_C 145.4, 111.9; δ_H 5.15 (1H, brs), 4.92 (1H, dd, J = 1.2, 1.2 Hz)], two hydroxymethyls [δ_C 66.8, δ_H 4.85 (1H, dd, J = 10.2, 3.0 Hz), 4.08 (1H, dd, J = 10.2, 10.2 Hz); δ_C 65.8, δ_H 3.83 (2H, d, J = 7.2 Hz)], two benzoyl moieties (δ_C 166.1, 168.4, 129.3, 130.8, 130.2 × 2, 129.6 × 2, 128.5 × 2, 128.4 × 2, 132.8, 133.6), an acetyl carbonyl resonance (δ_C 170.9), and, especially, a typical quaternary carbon resonance at δ_C 118.4 (C-1′), signifying that compound **3** is a 1α-alkyldaphnane derivative.[25,26] The NMR spectra of **3** resembled closely those of stelleralide A,[7] suggesting that the two compounds have the same molecular skeleton and substituent groups. However, detailed comparison of their ^{1}H and ^{13}C NMR data (Table 2) showed that an additional benzoyloxy group [δ_C 166.1 (Bz-CO), 130.8 (Bz-1″), 129.6 (Bz-2″ and 6″), 128.4 (Bz-3″ and 5″), and 132.8 (Bz-4″) and δ_H 8.06 (H-2″ and 6″), 7.45 (H-3″ and 5″), and 7.56 (H-4″)] is present in **3**. This conclusion was also supported by the molecular formula of **3**, which was 120 mass units greater than that of stelleralide A. The structure of **3** was fully determined by 2D NMR spectroscopic analysis. The HMBC correlations (Figure 1) from H-10′ (δ_H 1.22) to C-1 (δ_C 49.2) and C-9′ (δ_C 27.9) and from H-7′ (δ_H 5.34) to C-5′ (δ_C 19.3), C-9′ (δ_C 27.9), and a carbonyl group (δ_C 166.1) suggested that the benzoyloxy group is attached at C-7′. The relative configuration of **3** was established by its NOESY spectrum (Figure 1), comparison of its NMR data with those of stelleralide A, and molecular modeling and determined to be the same as that of stelleralide A with α-orientations of H-3, H-5, H-10, H-2′, H-7′, and H-10′ and β-orientations for H-1, H-8, H-7, H-14, and H-19. Consequently, compound **3** (stelleralide F) was assigned as shown.

Compound **4** was assigned the molecular formula $C_{51}H_{48}O_{14}$ based on a positive HRESIMS $[M + Na]^+$ ion at m/z 917.3694. The ^{1}H and ^{13}C NMR spectra of **4** and **3** were similar, suggesting that **4** is also a 1α-alkyldaphnane derivative. Detailed comparison of the ^{1}H and ^{13}C NMR data (Table 2) of **4** with those of **3** indicated the only difference to be an acetyloxy group at C-18 in **3** compared with a benzoyloxy group in **4**. This assignment was further confirmed by the HMBC correlation between H_2-18 (δ_H 4.41 and 5.11) and the benzoyloxy carbonyl (δ_C 166.5). The relative configuration of **4** was assigned as being the same as that of **3** based on comparison of their NMR data, NOESY experiments, and molecular modeling. Therefore, the structure of **4** (stelleralide G) was determined as shown.

Stelleralide H (**5**) was found to have the same molecular formula, $C_{44}H_{54}O_{12}$, as gnidimacrin (**10**), as determined by its HRESIMS. Detailed comparison of the NMR data of **5** with

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Table 2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Spectroscopic Data for Compounds 3−5 in $CDCl_3$

	3[a]		4[b]		5[c]	
position	δ_H [J in (Hz)]	δ_C	δ_H [J in (Hz)]	δ_C	δ_H [J in (Hz)]	δ_C
1	2.84 (1H, dd, 11.4, 11.4)	49.2	3.00 (1H, dd, 11.2, 11.2)	49.2	2.68 (1H, dd, 12.0, 12.0)	48.1
2	1.81 (1H, m)	37.4	1.87 (1H, m)	37.3	1.71 (1H, m)	37.4
3	4.90 (1H, d, 4.8)	82.4	4.96 (1H, d, 5.2)	82.3	3.81 (1H, d, 7.2)	79.5
4		79.4		79.5		78.5
5	4.06 (1H, d, 4.0)	73.5	4.12 (1H, d, 4.2)	73.2	3.79 (1H, s)	70.9
6		60.4		60.8		60.3
7	3.36 (1H, brs)	63.5	3.40 (1H, brs)	63.5	3.36 (1H, brs)	63.5
8	3.01 (1H, d, 1.8)	36.6	3.06 (1H, d, 1.8)	36.6	3.09 (1H, d, 1.8)	36.6
9		81.3		81.3		80.9
10	2.99 (1H, d, 12.0)	48.1	3.01 (1H, d, 12.0)	48.1	2.92 (1H, d, 14.4)	47.9
11	2.65 (1H, t, 7.8)	40.9	2.85 (1H, t, 7.8)	40.9	2.76 (1H, t, 7.8)	41.1
12	1.88 (1H, m)	29.2	1.98 (1H, m)	29.2	2.03 (1H, m)	29.5
	2.21 (1H, d, 12.0)		2.35 (1H, d, 14.8)		2.32 (1H, d, 14.4)	
13		84.4		84.5		84.5
14	4.37 (1H, d, 2.4)	81.3	4.43 (1H, d, 2.8)	81.3	4.40 (1H, d, 2.4)	81.4
15		145.4		145.4		145.6
16	4.93 (1H, dd, 1.2, 1.2)	111.9	4.98 (1H, dd, 1.2, 1.2)	111.7	4.97 (1H, 1.2, 1.2)	111.9
	5.15 (1H, s)		5.21 (1H, s)		5.20 (1H, s)	
17	1.80 (3H, s)	18.9	1.84 (3H, s)	18.8	1.84 (3H, s)	18.9
18	4.85 (1H, dd, 10.2, 3.0)	66.8	4.41 (1H, dd, 10.8, 3.8)	67.2	4.16 (1H, d, 12.0)	67.6
	4.08 (1H, dd, 10.2, 10.2)		5.11 (1H, dd, 8.0, 8.0)		5.01 (1H, d, 12.0)	
19	1.18 (3H, d, 6.6)	14.5	1.23 (3H, d, 6.8)	14.5	1.16 (3H, d, 6.0)	14.5
20	3.83 (2H, d, 7.2)	65.8	3.87 (2H, d, 7.2)	65.8	4.38 (1H, d, 10.2)	68.2
					4.91 (1H, d, 10.2)	
1′		118.4		118.4		118.5
2′	3.89 (1H, dd, 8.4, 3.0)	70.6	3.95 (1H, dd, 7.6, 3.0)	70.5	3.90 (1H, dd, 10.8, 3.6)	70.8
3′	1.56, 1.66 (1H, m)	28.6	1.59, 2.42 (1H, m)	28.6	1.71, 1.53 (1H, m)	28.5
4′	1.30, 1.66 (1H, m)	24.6	1.36, 1.66 (1H, m)	24.4	1.27, 1.58 (1H, m)	25.3
5′	1.40, 1.53 (2H, m)	19.3	1.48, 1.58 (2H, m)	19.3	1.20, 1.32 (2H, m)	24.2
6′	1.66, 1.87 (2H, m)	28.7	1.71, 1.93 (2H, m)	28.7	1.36 (2H, m)	23.6
7′	5.35 (1H, dd, 13.6, 6.8)	73.4	5.39 (1H, dd, 13.6, 6.8,)	73.4	1.24, 1.34 (1H, m)	23.1
8′	1.53, 1.96 (1H, m)	27.6	1.58, 2.01 (1H, m)	27.6	0.99, 1.59 (1H, m)	22.8
9′	2.31 (1H, m)	27.9	2.43 (1H, m)	28.1	2.28 (1H, m)	27.3
10′	1.22 (3H, d, 7.2)	18.5	1.30 (3H, d, 7.2)	18.5	0.99 (3H, d, 7.2)	18.2

[a]Data for benzoyl and acetyl groups: δ_H 8.19 (2H, dd, 7.8, 1.2, H-2″, H-6″), 7.48 (2H, dd, 6.8, 7.8, H-3″, 5″), 7.62 (1H, dd, 6.8, 1.2, H-4″), 8.06 (2H, dd, 10.4, 1.2, H-2‴, H-6‴), 7.45 (2H, dd, 10.4, 7.8, H-3‴, 5‴), 7.56 (1H, dd, 7.8, 1.0, H-4‴), 2.10 (3H, s, Ac-Me); δ_C 166.1, 168.4 (Bz-CO), 130.8 (C-1″), 130.2 (C-2″, 6″), 128.5 (C-3″, 5″), 133.6 (C-4″), 129.3 (C-1‴), 129.6 (C-2‴, 6‴), 128.4 (C-3‴, 5‴), 132.8 (C-4‴), 170.9 (Ac-CO), 21.1 (Ac-Me). [b]Data for benzoyl group: δ_H 8.19 (2H, dd, 12, 1.8, H-2″, H-6″), 7.39 (2H, dd, 12.0, 10.8, H-3″, 5″), 7.62 (1H, dd, 10.8, 1.8, H-4″), 8.09 (2H, dd, 10.8, 2.4, H-2‴, H-6‴), 7.48 (2H, dd, 10.8, 11.4, H-3‴, 5‴), 7.58 (1H, dd, 11.4, 2.4, H-4‴), 8.16 (2H, dd, 10.8, 1.8, H-2⁗, H-6⁗), 7.51 (2H, dd, 10.8, 12, H-3⁗, 5⁗), 7.54 (1H, dd, 12.0, 1.8, H-4⁗); δ_C 166.1, 166.5, 168.4 (Bz-CO), 129.3 (C-1″), 129.9 (C-2″, 6″), 128.4 (C-3″, 5″), 133.5 (C-4″),130.8 (C-1‴), 129.4 (C-2‴, 6‴), 128.5 (C-3‴, 5‴), 132.8 (C-4‴), 130.5 (C-1⁗),129.4, (C-2⁗, 6⁗), 128.6 (C-3⁗, 5⁗), 133.0 (C-4⁗). [c]Data for benzoyl group: δ_H 8.08 (4H, dd, 7.8, 1.2, H-2″, 6″, 2‴, 6‴), 7.46 (4H, dd, 7.8, 7.2, H-3″, 5″, 3‴, 5‴), 7.58 (2H, dd, 7.2, 1.2, H-4″, 4‴); δ_C 166.5, 167.1 (Bz-CO), 129.6 (C-1″), 129.9 (C-2″, 6″), 128.4 (C-3″, 5″), 133.1 (C-4″), 130.1 (C-1‴), 129.7 (C-2‴, 6‴), 128.4, 128.4 (C-3‴, 5‴), 133.1 (C-4‴).

those of **10** suggested that these compounds are structural analogues, differing only in the location of the benzoyl group. The benzoyl group was located at C-20 in **5** rather than at C-3 in **10** based on the HMBC correlation between H_2-20 (δ_H 4.38) and its corresponding benzoyl carbonyl (δ_C 167.5). The configuration of **5** was elucidated by NOESY correlations to be the same as that of **10**. Thus, the structure of **5** (stelleralide H) was established as shown.

Stelleralide I (**6**) was obtained as a white, amorphous powder, and its molecular formula was deduced as $C_{35}H_{50}O_9$ from an HRESIMS ion at m/z 637.3349 (calcd for $C_{35}H_{50}O_9Na$, 637.3347). The ^{1}H and ^{13}C NMR data (Table 3) of **6** were found to be closely similar to those of wikstroelide B (**14**), a daphnanetoxin diterpene. The main difference between them was the presence of a hydroxy group at C-12 in **6** rather than an acetyloxy group in **14**. This assignment was determined from the shifts in two carbon resonances: C-12 was shifted upfield from δc 78.2 in **14** to 77.6 in **6**, and C-13 was shifted downfield from δc 83.4 in **14** to δc 85.0 in **6**. The location of the hydroxy group at C-12 was confirmed by the HMBC correlations from H-12 (δ_H 3.94, 1H, brs) to C-9 (δ_C 78.5), C-11 (δ_C 44.9), C-13 (δ_C 85.0), C-14 (δ_C 80.6), and C-15 (δ_C 144.7). In addition, in the NOESY spectrum, H-12 correlated with H-18 (δ_H 1.23, 3H, d, J = 7.6 Hz), indicating that H-12 is α-oriented. Hence, the structure of compound **6** was fully established as shown.

Stelleralide J (**7**) was assigned a molecular formula of $C_{34}H_{52}O_{10}$, according to the HRESIMS (m/z 643.3455 [M + Na]$^+$, calcd for $C_{34}H_{52}O_{10}$, 643.3453). On the basis of its ^{1}H and ^{13}C NMR data (Table 3), **7** was considered to be a

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Table 3. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Spectroscopic Data for Compounds 6 and 7 in $CDCl_3$

	6		7	
position	δ_H [J in (Hz)]	δ_C	δ_H [J in (Hz)]	δ_C
1	7.59 (1H, brs)	160.9	7.73 (1H, s)	162.4
2		136.6		134.8
3		209.8		209.5
4		72.5		74.8
5	4.25 (1H, s)	72	3.83 (1H,s)	72.4
6		60.6		76.8
7	3.55 (1H, d, 1.8)	64.3	4.31 (1H, s)	79.1
8	3.78 (1H, brs)	34.9	3.29 (1H, brs)	40.1
9		78.5		76.3
10	3.85 (1H, t, 2.8)	47.6	3.63 (1H, d, 5.0)	53.3
11	2.49 (1H, dd, 14.0, 6.8)	44.9	2.24 (1H, m)	36.5
12	3.94 (1H, brs)	77.6	2.14 (2H, m)	37.5
13		85.0		74.4
14	4.74 (1H, d, 1.8)	80.6	5.65 (1H, s)	79.8
15		144.7		145.3
16	5.14 (2H, d, 10.8)	112.9	5.06 (1H, s), 5.17 (1H, s)	114.3
17	1.89 (3H, s)	18.9	1.88 (3H, s)	18.9
18	1.23 (3H, d, 7.6)	18.6	1.06 (3H, d, 8.5)	17.6
19	1.82 (3H, s)	9.9	1.80 (3H, s)	9.8
20	3.78 (1H, d, 12.0) 3.94 (1H, d, 12.0)	65.0	3.77 (2H, d, 11.2)	67.0
1′		116.8		168.6
2′	5.64 (1H, d, 15.2)	139.1	5.90 (1H, d, 16.0)	118.6
3′	6.67 (1H, dd, 15.2, 10.4)	122.7	7.35 (1H, dd, 14.0, 7.6)	146.8
4′	5.86 (1H, dd, 14.4, 8.0)	128.6	7.35 (1H, dd, 14.0, 7.6)	128.2
5′	5.86 (1H, dd, 14.4, 8.0)	134.9	6.21 (1H, dd, 14.0, 9.2)	146.3
6′	2.11 (2H, dd, 14.4, 7.2)	32.7	1.29 (2H, m)	31.9
7′	1.27 (2H, m)	29.2	1.30 (2H, m)	29.2
8′	1.28 (2H, m)	29.2	1.30 (2H, m)	29.2
9′	1.28 (2H, m)	29.3	1.30 (2H, m)	29.3
10′	1.29 (2H, m)	29.3	1.30 (2H, m)	29.4
11′	1.30 (2H, m)	29.5	1.30 (2H, m)	29.5
12′	1.30 (2H, m)	29.5	1.30 (2H, m)	31.8
13′	1.31 (2H, m)	31.9	1.30 (2H, m)	22.6
14′	1.31 (2H, m)	22.6	0.89 (3H, d, 6.4)	14.1
15′	0.90 (3H, t, 7.2)	14.1		

structural analogue containing 18 more mass units (H_2O) than wikstroelide M (**16**). A direct comparison of their ^{13}C NMR spectra showed that the carbon resonances at δ_C 61.9 (C-6) and 63.6 (C-7) in **16** were shifted downfield to δ_C 76.8 and 79.1 in **7**, suggesting that the 6,7-epoxide moiety in **16** is replaced by two hydroxy groups in **7**. This deduction was confirmed from the HSQC and HMBC spectra, particularly HMBC correlations (Figure 1) observed between H-7 (δ_H 4.31) and C-5 (δ_C 72.4), C-9 (δ_C 76.3), and C-20 (δ_C 67.0), as well as H-8 (δ_H 3.29) and C-6 (δ_C 76.8) (Figure 1). In the NOESY spectrum (Figure 1) of **7**, H-8 (δ_H 3.29) showed significant correlations with H-7 (δ_H 4.31) and H-14 (δ_H 5.65), suggesting that H-7, H-8, and H-14 have β-orientations. Correlation of H-7 (δ_H 4.31) and H_2-20 (δ_H 3.77) indicated that the hydroxymethyl at C-6 is also β-oriented, while the hydroxy at C-6 was α-oriented (Figure 1). A correlation was present between H-5 (δ_H 3.83) and H-10 (δ_H 3.63), but not between H_3-18 (δ_H 1.06) and H-8 (δ_H 3.29), which indicated that H-5, H-10, and H_3-18 have α-orientations. Consequently, compound **7** (Figure 1) was elucidated as shown.

The isolated compounds (**1**–**19**) were evaluated for anti-HIV activity against NL4-3 virus replication in MT4 lymphocytes. Cytotoxicity [50% toxic concentration (CC_{50})] was also assessed, and the results are summarized in Table 4.

Table 4. Anti-HIV and Cytotoxicity Activities of Compounds 1–19[a]

	anti-HIV	cytotoxicity	selectivity index
compound	EC_{50} (μM)	CC_{50} (μM)	CC_{50}/EC_{50}
1	0.59 ± 0.18	≥11.1	≥18
2	6.71 ± 2.46	≥10.8	≥1
3	0.00093 ± 0.00025	≥12	≥12903
4	0.00073 ± 0.00032	≥11.2	≥15342
5	0.00098 ± 0.00037	≥12.9	≥13163
6	0.12 ± 0.038	≥16.3	≥135
7	0.044 ± 0.015	≥4.4	≥100
8	0.33 ± 0.12	≥13.8	≥41
9	1.12 ± 0.30	≥18.3	≥16
10	0.00006 ± 0.00002	≥1.29	≥21500
11	0.0011 ± 0.0004	≥15.7	≥14272
12	0.048 ± 0.0180	≥15.3	≥318
13	0.012 ± 0.0041	≥15.6	≥1300
14	0.039 ± 0.0082	≥15.3	≥392
15	0.013 ± 0.0041	≥16.7	≥1284
16	0.0026 ± 0.0009	≥14	≥5384
17	0.059 ± 0.0150	≥16.6	≥281
18	0.044 ± 0.0118	≥15.2	≥345
19	0.047 ± 0.0148	≥18.8	≥400
AZT	0.032 ± 0.0082	≥3.74	≥116

[a]The values are means ± SD (n = 3). AZT (zidovudine) was used as a positive control.

The 1α-alkyldaphnane diterpenoids (**3**, **4**, **5**, **10**, and **11**) exhibited the most potent anti-HIV-1 activity, with EC_{50} values of 0.06–1.1 nM (SI > 10 000). The daphnanetoxin diterpenes (**6**, **7**, **13**–**19**) and compound **12** displayed lower, but still potent anti-HIV-1 effects (EC_{50} 2.6–120 nM, SI = 100–6000). The least potent compounds were **1**, **2**, **8**, and **9**, which contain a 2,4-epoxide moiety and lactone ring (SI < 50).

Structurally, the main difference between the most potent (**3**–**5**, **10**, **11**) and the least potent (**1**, **2**, **8**, **9**) compounds is in ring A. These results indicated that the nature of ring A appears to be responsible for the enhanced anti-HIV activity. In addition, compounds **3**–**5**, **10**, and **11** with a cyclopentane ring A were more potent than **6**, **7**, and **12**–**19** with a cyclopentenone or cyclopentanone ring A, suggesting the importance of a cyclopentane ring A for optimal anti-HIV activity.

On the basis of their significant anti-HIV activity, compounds **3**–**5**, **10**, and **11** should be investigated in greater detail to develop a deeper understanding of their anti-HIV characteristics and potential. The new compounds likely share the same mechanisms of action as previously investigated daphnane- and tigliane-type diterpenes.[7,10,27]

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on an Autopol V Plus instrument (Rudolph, Hackettstown, NJ, USA). UV spectra were recorded in MeOH using a Lambda 25

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spectrophotometer (PerkinElmer, Wellesley, MA, USA). CD spectra were obtained on a JASCO J-715 spectrometer. IR spectra were measured on a PE Spectrum RXI spectrophotometer (PerkinElmer) using KBr pellets. NMR spectroscopic data were recorded at room temperature on Bruker AMX-400 MHz and AMX-600 MHz instruments in $CDCl_3$ with TMS as an internal standard. Standard pulse sequences were employed for the measurement of 2D NMR spectra ($^1H-^1H$ COSY, HSQC, HMBC, and NOESY). ESIMS analysis were carried out on a Dionex Ultimate 3000 UPLC instrument with an LTQ Velos Pro MS spectrometer (Thermo Fisher Scientific, USA). HRESIMS were acquired with a Bruker Daltonics APEXIII 7.0 TESLA FTMS system (Bruker Daltonics, Billerica, MA, USA). Analytical HPLC was carried out on an Agilent 1200 series LC instrument with a DAD detector (Agilent Technologies, Palo Alto, CA, USA) and a Symmetry C_{18} column (4.6 × 250 mm, 5 μm). Preparative HPLC was performed on an Agilent 1100 (Agilent Technologies) and a YMC-Pack Pro C_{18} RS column (20 × 250 mm, 5 μm). Silica gel (200−300 mesh, Qingdao Haiyang Chemical Co. Ltd., Qingdao, People's Republic of China), Sephadex LH-20 (25−100 μm, Pharmacia, Germany), and RP-C_{18} (30−50 μm, Fuji Silysia Chemical Co. Ltd., Aichi, Japan) were used for column chromatography (CC). The fractions were monitored by TLC (HSGF 254, Yantai, People's Republic of China), and detection was achieved by 10% H_2SO_4 in EtOH. All solvents used for CC were of analytical grade (Shanghai Chemical Reagents Co. Ltd., Shanghai, People's Republic of China), and solvents used for HPLC were of HPLC grade. Linoleic acid was obtained from Sigma-Aldrich Company Ltd., Gillingham, United Kingdom.

Plant Material. The roots of *S. chamaejasme* (4 years old) were purchased from Baotou, Inner Mongolia, People's Republic of China, in August 2011 and were authenticated by one of the authors (D.-F.C.). A voucher specimen (DFC-YM-SC-2011-08) is deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, People's Republic of China.

Extraction and Isolation. The dried roots of *S. chamaejasme* were ground into a powder (40 kg), which was percolated with 95% aqueous EtOH. After removal of the solvent under vacuum, the residue was suspended in H_2O and successively extracted with petroleum ether, EtOAc, and *n*-BuOH. The petroleum ether-soluble fraction (350 g) was subjected to VLC on silica gel using a stepwise gradient elution of petroleum ether−Me_2CO (30:1, 20:1, 10:1, 5:1, 2:1, and 1:1) to afford five subfractions (Fr.A−Fr.E). Fr.C (40 g) was passed through a silica gel column eluted with petroleum ether−EtOAc (30:1 to 10:1) to give seven fractions (Fr.C1−Fr.C7). Fr.C2 (7 g) was applied to CC on Sephadex LH-20 (*n*-hexane−CH_2Cl_2−MeOH, 5:4:1) to obtain six fractions (Fr.C2a−Fr.C 2f). Fr.C2b (120 mg) was purified by preparative HPLC (10 mL/min, 50 min 85−95% MeCN−H_2O gradient elution) to yield **1** (10 mg) and **2** (8 mg). Fr.C2f (400 mg) was separated by preparative HPLC (10 mL/min, 50 min 70−95% MeCN−H_2O gradient elution) to acquire **12** (15 mg), **13** (35 mg), and **16** (40 mg). Fr.C3 (5 g) was chromatographed on an RP-C_{18} silica gel column (MeOH−H_2O, 70:30 to 100:0) to give five fractions (Fr.C3a−Fr.C3e). Fr.C3d (1.2 g) was separated on a preparative HPLC column (10 mL/min, 75% MeCN−H_2O isocratic elution) to obtain **3** (8 mg), **4** (5 mg), **9** (20 mg), **10** (15 mg), and **19** (25 mg). Separation of Fr.D (30 g) by MPLC (petroleum ether−EtOAc, 15:1 to 0:1) gave six fractions (Fr.D1−Fr.D6). Fr.D3 was purified by passage over Sephadex LH-20 ($CHCl_3$−MeOH, 1:1) and then by preparative HPLC (CH_3CN-H_2O, eluting from 65:35 to 90:10 for 40 min with a flow rate of 10 mL/min) to afford **6** (4 mg), **7** (7 mg), **8** (25 mg), **17** (12 mg), and **15** (9 mg). Using the same purification procedures, Fr.D4 afforded **5** (9 mg), **11** (11 mg), **18** (7 mg), and **14** (20 mg).

*Stelleralide D (**1**):* colorless oil; $[\alpha]_D^{20}$ +5.3 (*c* 0.10, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ε) 233 (4.38) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 208 (4.06), 228 (7.70); IR (KBr) ν_{max} 3478, 2924, 2854, 1799, 1718, 1648, 1452, 1396, 1270, 713 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 1; HRESIMS *m/z* 927.5235 $[M + Na]^+$ (calcd for $C_{53}H_{76}O_{12}Na$, 927.5229).

*Stelleralide E (**2**):* colorless oil; $[\alpha]_D^{20}$ −4.8 (*c* 0.10, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ε) 233 (4.16) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 208 (4.67), 228 (7.78); IR (KBr) ν_{max} 3459, 2923, 2854, 2359, 2341, 1797, 1717, 1455, 1395, 1173, 717 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 1; HRESIMS *m/z* 951.5184 $[M + Na]^+$ (calcd for $C_{55}H_{76}O_{12}Na$, 951.5229).

*Stelleralide F (**3**):* white, amorphous powder; $[\alpha]_D^{20}$ −7.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 233 (4.02) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 215 (1.11), 236 (−1.14), 273 (0.87); IR (KBr) ν_{max} 3463, 2925, 2860, 2360, 2340, 1731, 1712, 1457, 1384, 1279, 1025, 713 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2; HRESIMS *m/z* 855.3557 $[M + Na]^+$ (calcd for $C_{46}H_{56}O_{14}Na$, 855.3562).

*Stelleralide G (**4**):* white, amorphous powder; $[\alpha]_D^{20}$ −16.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 233 (4.15) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 215 (1.07), 237 (−7.42); IR (KBr) ν_{max} 3453, 2934, 2854, 1715, 1451, 1384, 1275, 1025, 712 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2; HRESIMS *m/z* 917.3694 $[M + Na]^+$ (calcd for $C_{51}H_{48}O_{14}Na$, 917.3719).

*Stelleralide H (**5**):* white, amorphous powder; $[\alpha]_D^{20}$ +4.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 233 (4.31) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 215 (4.07), 236 (−5.42); IR (KBr) ν_{max} 3446, 2927, 2858, 1716, 1451, 1272, 1112, 1023, 711 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2; HRESIMS *m/z* 775.3693 $[M + H]^+$ (calcd for $C_{44}H_{54}O_{12}$ 775.3688).

*Stelleralide I (**6**):* white, amorphous powder; $[\alpha]_D^{20}$ +21.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 256 (3.98) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 226 (−12.1), 242 (5.82); IR (KBr) ν_{max} 3435, 2920, 2843, 1709, 1616, 1463, 1375, 1019 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 3; HRESIMS *m/z* 637.3349 $[M + Na]^+$ (calcd for $C_{35}H_{50}O_9Na$, 637.3347).

*Stelleralide J (**7**):* white, amorphous powder; $[\alpha]_D^{20}$ +2.2 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 256 (4.01) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 243 (−1.69), 268 (3.08); IR (KBr) ν_{max} 3405, 2925, 2854, 2361, 1701, 1639, 1006 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 3; HRESIMS *m/z* 643.3455 $[M + Na]^+$ (calcd for $C_{34}H_{50}O_{10}Na$, 643.3453).

Base Hydrolysis of 2 and GC-MS Analysis. Compound **2** (2 mg) in CH_2C1_2 (2 mL) was allowed to stand at room temperature for 3 h with 0.05 M NaOMe in MeOH (0.5 mL). The mixture was neutralized, followed by addition of 10 mL of 14% BF_3−MeOH, and was heated at 80 °C for 5 min. Hexane (3 mL) was added to the above mixture through the top of the condenser, and heating was continued for 2 min. After dilution with a saturated NaCl solution, the organic layer was collected and evaporated to dryness using N_2. The residue was redissolved in hexane and analyzed by GC-MS (Shimadzu, GCMS-QP2010 Ultra) using an Inertcap column (0.25 mm × 30 m) under the following conditions [injector temperature, 250 °C; initial temperature, 80 °C (1 min), increased at 25 °C/min to 230 °C, held for 10 min; carrier gas, He operated in the splitless mode; injection size, 0.2 μL; MS conditions: EI voltage, 70 eV; scanned-mass range, *m/z* 50−1000]. Identification of linoleic acid was carried out for **2**, giving a peak at 12.23 min. With authentic linoleic acid, a peak was detected at 12.24 min.

Anti-HIV Assays. HIV-1 NL4-3 (multiplicity of infection = 0.001) was used to infect MT4 cells in the presence of various concentrations of compounds. Fresh medium, which contained appropriate concentrations of the compounds, was added to the culture 48 h after infection to maintain normal cell growth. Virus replication was analyzed on day 4 postinfection using p24 ELISA kits from PerkinElmer. The compound concentration that inhibited HIV-1 replication by 50% (EC_{50}) was calculated by using the biostatistics software CalcuSyn (Biosoft).

Cytotoxicity Assays. Cytotoxicity of the purified compounds toward MT4 cells was determined by using a cell viability kit provided by Promega. The CellTiter-Glo luminescent cell viability assay is a simple method of determining the viability of the cells in culture based on quantitation of ATP in metabolically active cells. The CellTiter-Glo

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reagent was added to MT4 cells that were cultured parallel to the antiviral assays. The cytotoxic concentration that caused the reduction of viable cells by 50% (CC_{50}) was calculated from the dose−response curve using CalcuSyn.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00660.

^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, HSQC, HMBC, NOESY, CD, and IR spectra for compounds **1**−**7** (PDF)

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Notes

The authors declare no competing financial interest.

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