Bioorganic & Medicinal Chemistry Letters 21 (2011) 961–965

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Compounds from *Kadsura angustifolia* with anti-HIV activity

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ARTICLE INFO

Article history:
Received 31 August 2010
Revised 23 November 2010
Accepted 9 December 2010
Available online 21 December 2010

Keywords:
Kadsura angustifolia
Triterpenoid
Angustific acid
Angustifodilactone
Anti-HIV

ABSTRACT

Four new cycloartane triterpenoids, angustific acid A (**1**), angustific acid B (**2**), angustifodilactone A (**3**) and angustifodilactone B (**4**) were isolated from the branches of *Kadsura angustifolia* together with six known compounds, micranoic acid B (**5**), nigranoic acid (**6**), schisandrin (**7**), schisantherin D (**8**), interiotherin B (**9**), schisantherin B (**10**). Their structures were established on the basis of extensive spectroscopic data analyses and comparison with spectroscopic data reported. Compound **1**, characterized by the presence of a C-16/C-17, C-20/C-21 conjugated diene and a C-1/C-7 ester bridge formed in rings A and B, provided a novel structural skeleton for 3,4-secocycloartane triterpenoid derivatives. In addition, the anti-HIV activities of these compounds were determined in infected C8166 cells, and it was found that angustific acid A (**1**) exhibited the most potent anti-HIV activity with an EC_{50} value of 6.1 μg/mL and a therapeutic index of more than 32.8.

A considerable number of studies have been performed on plants of the family Schisandraceae, which contains only two genera, *Schisandra* and *Kadsura*.[1–8] These investigations have yielded dibenzocyclooctadiene lignans, as well as lanostane and cycloartane triterpenes, some of which have been found to possess many beneficial pharmacological properties, including anti-HIV, antitumor, and antihepatitis activities and inhibitory activity on cholesterol biosynthesis.[1–8] *Kadsura angustifolia* (Lem.) Smith is an evergreen liana, growing in the forests at elevations of 1280–2250 m in Yunnan Province, China (*Flora Yunnanica;* Science Press: Beijing, 2000; Vol. 11, p 16.). Its branches are used as a folk medicine to promote blood circulation and treat fractures and menstrual irregularities. Some lignans and triterpenoids were isolated from this species in past decades.[9–11] In the course of a search for bioactive natural products, we have investigated this plant and isolated four new cycloartane triterpenoids, angustific acid A (**1**), angustific acid B (**2**), angustifodilactone A (**3**) and angustifodilactone B (**4**), together with six known compounds, micranoic acid B (**5**),[12] nigranoic acid (**6**),[3,13] schisandrin (**7**),[14] schisantherin D (**8**),[15] interiotherin B (**9**),[16] and schisantherin B (**10**)[17] (Fig. 1). Compound **1** was found to possess an unusual C-16/C-17, C-20/C-21-diene structure and a unique ester bridge between C-1 and C-7 in rings A and B, which provided a novel structural skeleton for 3,4-secocycloartane triterpenoid derivatives. In addition, all compounds were tested for their anti-HIV activity. Thus, the structure elucidation of the four new natural products **1**–**4** and the anti-HIV activities of compound **1**–**10** are discussed herein.

The air-dried branches of *K. angustifolia* (5 kg) collected in September 2007 from Wenshan Prefecture of Yunnan Province, China, were powdered and extracted with 70% aqueous Me_2CO (4 × 20 L) at room temperature and concentrated in vacuo to give a crude extract, which was partitioned between H_2O and EtOAc. The EtOAc-soluble portion (165 g) was repeatedly subjected to CC chromatography over MCI, silica gel and Sephadex LH-20 to yield compounds **1**–**10**.[18]

Angustific acid A (**1**)[19] was obtained as light yellow oil (CH_3COCH_3) and possessed the molecular formula $C_{30}H_{40}O_4$, derived from its HREIMS analysis (*m/z* 464.2933 $[M]^+$, calcd, 464.2927), indicating 11 degrees of unsaturation. The ^1H NMR spectrum (Table 1) showed four methyls [two tertiary methyls at δ_H 1.11 (CH_3-30) and 1.28 (CH_3-18), and two olefinic methyls at δ_H 1.87 (CH_3-26) and 1.70 (CH_3-28)], an oxymethine at δ_H 3.60 (1H, m), two olefinic methylenes [δ_H 4.74/4.93 and 4.89/5.07, Ha-28/Hb-28, Ha-21/Hb-21, respectively], two olefinic methines [δ_H 6.04 (1H, t, J = 7.3 Hz) and 5.76 (1H, br s)] and one pair of typical cycloartane methylene protons [δ_H 0.94 and 0.47 (each 1H, d, J = 4.0 Hz)]. The ^{13}C, in combination with DEPT experiments, resolved 30 carbon resonances attributable to two carbonyls, four sp^2 quaternary carbons, two sp^2 methines, two sp^2 methylenes, four sp^3 quaternary carbons, three sp^3 methines, nine sp^3 methylenes and four methyls (Table 1). The presence of these features revealed that a total of 39 protons were attached to carbons,

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doi:10.1016/j.bmcl.2010.12.055

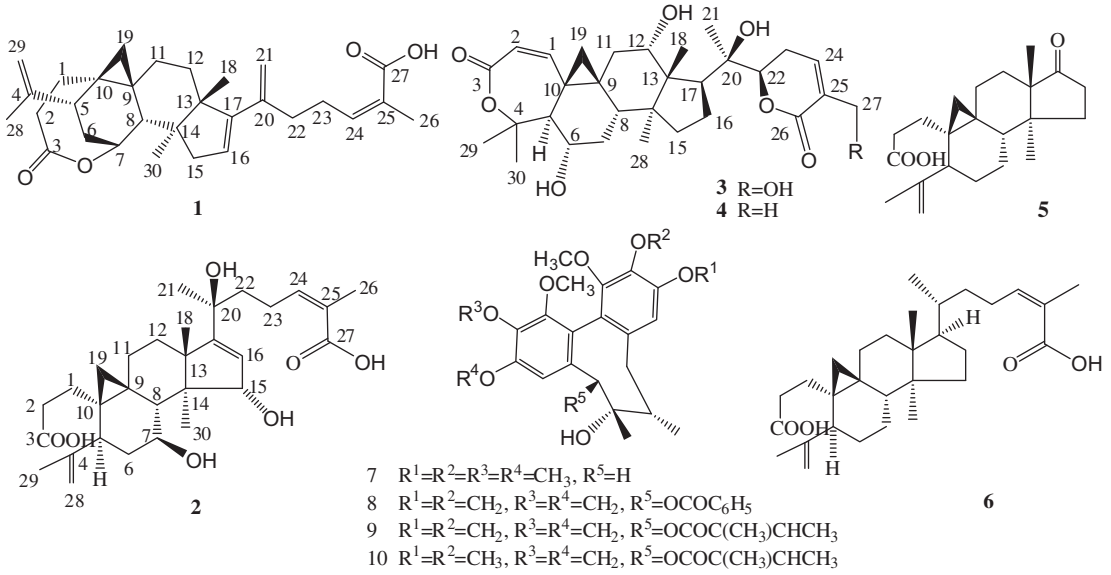

Figure 1. The structures of compound **1**–**10** isolated from *K. angustifolia*.

implying the presence of only one exchangeable proton in the molecule of **1**. This also suggested that compound **1** is pentacyclic.

A complete analysis of a combination of the ^{1}H, ^{13}C, DEPT, HMQC, COSY and HMBC NMR spectra suggested that compound **1** has the same 3,4-secocycloartane skeleton as a coexisting major triterpenoid, nigranoic acid (**6**)[3] which was supported by salient HMBC correlations of H_2-1 with C-3, H_2-2 with C-3, H_2-19 with C-1, C-5, C-8, C-9, C-10 and C-11, H-5 with C-1 and C-28, C**H**$_3$-29 with C-4, C-5 and C-28, C**H**$_3$-26 with C-24, C-25 and C-27, H-24 with C-22 and C-26, and H_2-23 with C-20, C-22 and C-25 along with the ^{1}H–^{1}H COSY spin systems of H_2-1/H_2-2 and H_2-22/H_2-23/H-24/C**H**$_3$-26. However, the distinct differences between two compounds in NMR spectroscopic data were the appearance of an oxymethine resonance [δ_H 3.60 (1H, m, H-7); δ_C 69.6 (d)] and four more olefinic resonances [δ_H 4.89 (1H, s, Ha-21), 5.07 (1H, s, Hb-21) and 5.76 (1H, br s, H-16); δ_C 128.4 (d, C-16); 150.5 (s, C-17), 144.1 (s, C-20) and 110.8 (t, C-21)] in **1**. The positions of each functional group were determined by the HMBC and ^{1}H–^{1}H COSY NMR experiments. Thus, the ^{1}H–^{1}H COSY correlation of H-16/H_2-15 and HMBC correlations of H_2-21 with C-17, C-20 and C-22, H-16 with C-13, C-14, C-15, C-17 and C-20, C**H**$_3$-18 with C-12, C-13, C-14 and C-17, and C**H**$_3$-30 with C-8, C-13, C-14 and C-15 confirmed the presence of a trisubstituted olefin at C-16/C-17 and a 1,2-disubstituted olefin at C-20/C-21, which yielded an unusual conjugated diene structure in triterpenoid **1**. Furthermore, the oxymethine proton at δ_H 3.60 were determined to be at H-7 based on the ^{1}H–^{1}H COSY correlations of H-5/H_2-6/H-7/H-8 and HMBC correlations of H-5, H-8, H_2-6 with C-7 (δ_C 69.6, d), and H-7 with C-5, C-8 and C-14. Finally, proton H-7 also showed a key HMBC correlation with the carboxyl carbon C-3, suggesting an ester linkage between C-3 and C-7 in **1**, which yielded a modified nine-membered lactone ring that has never been reported previously in triterpenoids. On the basis of these data, the gross structure of **1** was elucidated as shown.

The relative stereochemistry of compound **1** was determined using information from NOESY spectrum and by comparison of its spectroscopic data to those of nigranoic acid.[3] The *E*-geometry of the C-17/C-20 olefin in **1** was determined by the NOESY correlation of Hb-21 with C**H**$_3$-18 and H_2-12, Ha-21 with H_2-22, and H-16 with H_2-23. Since no cross-peak was observed between H-7 and H-8 in the NOESY spectrum, H-7 was assigned to be α-oriented, which was further confirmed by the strong NOESY correlations of H-7 with H_α-6, H-5 and C**H**$_3$-30. On the basis of a computer-generated 3D structure obtained by CHEM3D ULTRA V 8.0, with MM2 force-field calculations for energy minimization, if H-5 has the same α-orientation as nigranoic acid, the interatomic distance between H-5 and H-7 would be approximately 4.317 Å, so the NOESY correlation of H-5 with H-7 could not be observed. However, the correlation between H-5 and H-7 was clearly observed in the NOESY spectrum, which indicated that H-5 in **1** is β-oriented. This was further confirmed by the observed NOESY correlations of H-5 with H_β-19, Hb-28, C**H**$_3$-29 and H-8. Finally, the NOESY correlations of H_β-19 with C**H**$_3$-29, C**H**$_3$-18 and H-8, H-8 with C**H**$_3$-18, C**H**$_3$-30 with H_α-11 and H_α-12, and H-24 with C**H**$_3$-26 suggested that other chiral centers in **1** were the same as those of **6**. Additionally, according to the above computer-generated 3D structure, the calculated interatomic distances between H-7/C**H**$_3$-30 (2.086 Å), H-7/H-5 (3.535 Å), H-7/H_α-6 (2.385 Å), Hb-21/C**H**$_3$-18 (2.081 Å), Hb-21/H_2-12 (2.539, 3.441 Å), Ha-21/H_2-22 (2.369, 2.935 Å), H-16/H_2-23 (2.203, 2.449 Å), H-5/H_β-19 (2.394 Å), H-5/H_b-28 (2.600 Å), H-5/C**H**$_3$-29 (3.053 Å), H-5/H-8 (1.890 Å), H_β-19/C**H**$_3$-29 (2.578 Å), H_β-19/C**H**$_3$-18 (2.313 Å), H_β-19/H-8 (2.938 Å), H-8/C**H**$_3$-18 (2.394 Å), C**H**$_3$-30/H_α-11 (2.095 Å), C**H**$_3$-30/H_α-12 (2.529 Å) and H-24/C**H**$_3$-26 (2.333 Å) are all less than 3.60 Å (Fig. 3). This further supported the well-defined NOESY correlations observed for each of these proton pairs. Thus, the structure of **1**, named angustific acid A, was unambiguously determined as 3,4-secocycloarta-4 (28), 16 (17), 20 (21), 24-(*Z*)-tetraene-3,7β,-lactone-3-oic acid. This structure is substantially different from A secocycloartane terpenords so far isolated, since C-7 was lactonized to C-3 and H-5 was β-oriented.

Angustific acid B (**2**)[20] gave two ion peaks at *m/z* 539 $[M + Na]^+$ and 1056 $[2M + Na+1]^+$ in its positive ESIMS spectrum and was assigned a molecular formula of $C_{30}H_{44}O_7$ (nine degrees of unsaturation), which was confirmed by HRESIMS (found $[M + Na]^+$ *m/z* 539.2987, calcd 539.2984) and the NMR data (Table 1). Comparison of the spectroscopic data of **2** with those of **6**[3] revealed that they were quite similar except for the presence of more oxygenated and olefinic carbons [δ_H 3.47 (1H, m, H-7), 4.55 (1H, br s, H-15) and 5.34 (1H, br s, H-16); δ_C 69.1 (d, C-7), 74.1 (s, C-20), 80.9 (d, C-15), 126.5 (d, C-16) and 156.7 (s, C-17)] in **2**. The positions of each functional group were determined by the HMBC and ^{1}H–^{1}H COSY NMR experiments (Fig. 2). Because of the absence of NOESY correlation between C**H**$_3$-18 and C**H**$_3$-21, the relative configuration of OH-20 (δ_H 5.32) in **2** was assigned to be β-oriented

Table 1
The NMR data of compounds **1–3** in CD_3COCD_3 and **4** in pyridine-d_5

No.	1		2		3		4	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.31 (m) 2.52 (m)	31.4 t	2.18 (m) 2.56 (m)	31.5 t	6.09 (d, 12.6)	152.1 d	6.19 (m)	152.0 d
2	2.01 (m) 1.48 (m)	30.5 t	1.41 (m) 2.08 (m)	29.8 t	5.87 (d, 12.6)	120.0 d	6.20 (m)	119.8 d
3		174.8 s		174.8 s		167.0 s		167.2 s
4		149.4 s		149.4 s		84.7 s		84.8 s
5	2.75 (dd, 4.0, 13.0)	45.7 d	2.78 (dd, 4.2, 13.1)	45.7 d	2.26 (d, 3.8)	49.4 d	2.28 (overlapped)	49.1 d
6	1.28 (m) 1.62 (m)	38.1 t	1.65 (m) 1.38 (m)	36.8 t	4.45 (br s)	66.6 d	4.48 (br s)	65.9 d
6-O**H**					3.85 (br s)			
7	3.60 (m)	69.6 d	3.47 (m)	69.1 d	1.61 (m) 1.46 (m)	33.4 t	1.62 (m) 1.29 (m)	33.4 t
8	1.87 (overlapped)	53.9 d	1.86 (overlapped)	55.2 d	2.27 (overlapped)	39.3 d	2.50 (dd, 12.3, 6.8)	39.0 d
9		21.2 s		20.9 s		28.6 s		28.4 s
10		29.7 s		28.3 s		32.6 s		32.3 s
11	1.48 (m) 2.22 (m)	27.6 t	1.38 (m) 2.20 (m)	27.5 t	1.45 (m) 2.79 (m)	42.8 t	1.74 (d, 15.4) 2.89 (dd, 6.4, 15.1)	43.0 t
12	1.95 (m) 1.98 (m)	28.8 t	1.89 (m) 2.17 (m)	28.8 t	4.10 (t, 6.3)	72.6 d	4.56 (d, 7.1)	72.2 d
12-O**H**					3.90 (br s)			
13		52.4 s		53.4 s		51.6 s		49.7 s
14		49.8 s		54.2 s		50.1 s		51.4 s
15	2.38 (m) 2.19 (m)	45.2 t	4.55 (br s)	80.9 d	1.40 (m) 1.47 (m)	35.8 t	1.30 (m) 1.48 (m)	35.5 t
16	5.76 (br s)	128.4 d	5.34 (br s)	126.5 d	1.91 (m)	23.7 t	2.10 (m)	23.8 t
17		150.5 s		156.7 s	2.76 (overlapped)	53.1 d	3.26 (t, 9.8)	52.6 d
18	1.28 (s)	22.8 q	1.39 (s)	24.7 q	1.14 (s)	13.7 q	1.44 (s)	13.9 q
19	0.94 (d, 4.4, βH) 0.47 (d, 4.4, αH)	31.2 t	0.94 (d, 4.3, βH) 0.50 (d, 4.3, αH)	32.6 t	2.05 (d, 3.8, βH) 1.27 (d, 3.8, αH)	37.6 t	2.33 (overlapped) 1.29 (d, 3.8, βH)	37.5 t
20		144.1 s		74.1 s		75.6 s		75.3 s
20-O**H**			5.32 (s)		3.64 (s)			
21	4.89 (s) 5.07 (s)	110.8 t	1.41 (s)	24.8 q	1.25 (s)	21.4 q	1.47 (s)	21.4 q
22	2.38 (m) 2.30 (m)	36.4 t	1.70 (m) 1.84 (m)	43.2 t	5.10 (dd, 12.6, 3.7)	82.9 d	5.63 (dd, 12.9, 3.1)	83.1 d
23	2.67 (m)	29.8 t	2.59 (m)	25.3 t	2.50 (m) 2.58 (m)	25.4 t	2.72 (m) 2.28 (m)	25.5 t
24	6.04 (t, 7.3)	143.2 d	6.01 (t, 7.0)	143.4 d	6.93 (dd, 1.7, 6.6)	139.9 d	6.44 (d, 7.2)	140.7 d
25		127.9 s		128.0 s		132.4 s		127.7 s
26	1.87 (s)	21.2 q	1.86 (s)	21.0 q		165.1 s		166.5 s
27		169.1 s		169.4 s	4.19 (d, 5.29) 4.10 (br s)	60.3 t	1.81 (s)	17.0 q
27-O**H**								
28	4.93 (s) 4.74 (s)	112.1 t	4.75 (s) 4.91 (s)	19.9 q	1.00 (s)	20.3 q	0.94 (s)	20.0 q
29	1.70 (s)	19.8 q	1.71 (s)	112.2 t	1.45 (s)	24.4 q	1.60 (s)	24.9 q
30	1.11 (s)	20.6 q	1.02 (s)	13.4 q	1.60 (s)	28.3 q	1.99 (s)	28.6 q

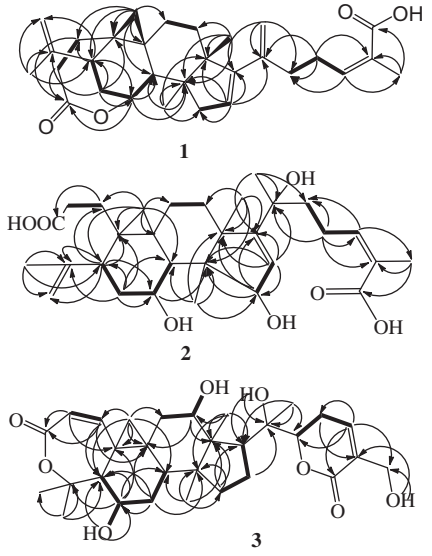

Figure 2. Key HMBC (→) and COSY (▬) correlations for compounds **1–3**.

which also confirmed by the NOESY correlation of H-16 with C**H**$_3$-21 and H$_2$-22, O**H**-20 with H-23. Furthermore, the NOESY correlations of H-15 with H-8, H-16 and C**H**$_3$-18, and H-7 with H$_\alpha$-6, C**H**$_3$-30 and H-5 indicated that H-15 was β-oriented while H-7 was α-oriented. Finally, the NOESY correlations of H-5 with H$_b$-28, H$_\beta$-19 with H-8, C**H**$_3$-18, C**H**$_3$-29 and H$_\beta$-6, C**H**$_3$-30 with H$_\alpha$-12, and C**H**$_3$-26 with H-24 suggested that other chiral centers in **2** were the same as those of **6**. Thus, the structure of **2**, named angustific acid B, was unambiguously determined as 3,4-secocycloarta-4 (28), 16,24-(*Z*)-triene-7β,15α,20β-trihydroxy-3-oic acid.

Angustifodilactone A (**3**),[21] obtained as colorless solid, was found to possess a molecular formula of $C_{30}H_{42}O_8$, as evidenced by HRMS (ESI-TOF) (*m*/*z* 553.2788 [M + Na]$^+$, calcd 553.2777) and ^{13}C NMR spectroscopic data, requiring 10 degrees of unsaturation. Analysis of ^{1}H, ^{13}C and HMQC NMR data for **3** revealed the presence of four exchangeable protons, five tertiary methyls, seven sp^3 methylenes, six sp^3 methines, six sp^3 quaternary carbons, four olefinic carbons and two α,β-unsaturated carbonyl carbons (Table 1). A complete analysis of a combination of the ^{1}H, ^{13}C, DEPT, HMQC, COSY and HMBC NMR spectra suggested that compound **3** has the same cycloartane triterpene dilactone skeleton as the

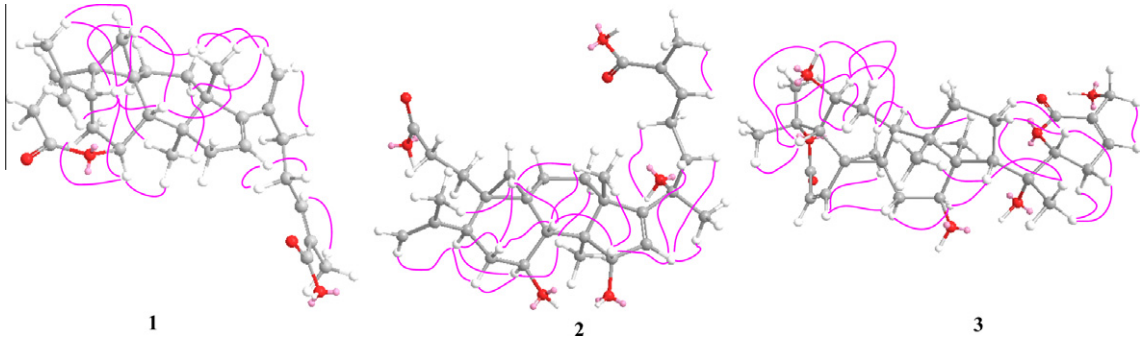

Figure 3. Key NOESY correlations for compounds **1**–**3** and conformations generated from computer modeling (ChemDraw. 9.0.3D).

Table 2
Summary of cytotoxicities and anti-HIV-1 activities of compounds **1**–**10**

Compound	Cytotoxicity, CC_{50} (μg/mL)	HIV-1_{IIIB} activity, EC_{50} (μg/mL)	Therapeutic index CC_{50}/EC_{50}
1	>200	6.11	>32.8
2	156.2	11.2	13.9
3	178.2	13.3	13.4
4	182.1	13.6	13.4
5	176.1	15.7	11.2
6	89.0	10.5	8.48
7	>200	15.8	>12.66
8	>200	20.5	>9.76
9	>200	19.0	>10.53
10	>200	22.1	>9.05
AZT	>200	0.0033	>60606.1

known *Kadsura* nortriterpenoid, kadsuphilactone B.[22] Differences between two compounds in ^1H and ^{13}C NMR spectroscopic data were the absence of a methyl [δ_H 1.90 (s, CH_3-27); δ_C 16.8 (q, C-27)] and two methylenes [δ_H 0.79/1.87 (each, m, H_2-6) and 2.10/1.61 (each, m, H_2-12); δ_C 24.1 (t, C-6) and 33.0 (t, C-12)] in kadsuphilactone B[22] and the presence of an oxygenated methylene [δ_H 4.19 (d, J = 5.3 Hz, H_2-27); δ_C 60.3 (t, C-27)], and two oxygenated methines in **3** [δ_H 4.45 (br s, H-6) and 4.10 (t, J = 6.3 Hz, H-12); δ_C 66.6 (d, C-6) and 72.6 (d, C-27)], thus suggesting that C-6, C-12 and C-27 of **3** were each attached to a hydroxyl group. This was further confirmed by the HMBC and ^1H–^1H COSY NMR experiments (Fig. 2). The *R*-configuration of the chiral center at C-22 of **3** was determined on the basis of the positive Cotton effect at 279 nm, similar to that of kadsuphilactone B.[22,23] From the moderate vicinal coupling constant values (<8 Hz) between H_2-7/H-6 and H_2-11/H-12, both hydroxyl groups attached to C-6 and C-12 were assigned to be in an α-orientation, which were further confirmed by the NOESY correlation of H-6 with H_2-7, H_β-19 and H-5, O**H**-6 with H_2-7 and H-5, and H-12 with H_α-11 and H-17. Finally, the NOESY correlations of C**H**$_3$-30 with H-5, H_β-19 with H-8, H-1 with H_α-19 and H_2-11, H-8 with C**H**$_3$-18, C**H**$_3$-28 with H-17, H-17 with C**H**$_3$-21, H-16 with H-22 and C**H**$_3$-21, C**H**$_3$-21 with H_2-23, and H-24 with H_2-27 suggested that other chiral centers in **3** were the same as those of kadsuphilactone.[22] Thus, the structure of **3**, named angustifodilactone A, was unambiguously determined as (5*R*, 6*S*, 8*S*, 9*S*, 10*R*, 12*S*, 13*S*, 14*S*, 17*S*, 20*R*, 22*R*)-3,4-secocycloarta-1,24-diene-6,12,20,27-tetrahydroxy-3,4-lactone-22,26-lactone.

Angustifodilactone B (**4**)[24] was assigned a molecular formula of $C_{30}H_{42}O_7$ on the basis of its HRESIMS (*m/z* 537.2825 $[M + Na]^+$) and NMR data (Table 1). Analysis of the ^1H, ^{13}C and HMQC NMR data for **4** revealed the presence of structural features similar to those found in **3**, except that the hydroxymethyl group (δ_H 4.19; δ_C 60.3) was replaced by the signal for an olefinic methyl unit (δ_H 1.81; δ_C 17.0) in the NMR spectra of **4**. This was further confirmed by HMBC correlations from the new olefinic methyl protons (δ_H 1.81) to C-24, C-25 and C-26. Therefore, the structure of **4** was established. Similar NOEs to those observed for **3** pointed to the same stereochemistry.

Since some nortriterpenoids and lignans isolated from plants of the family Schisandraceae are reported to possess anti-HIV activities,[2] compounds **1**–**10** were tested for cytotoxicity assay against C8166 cells (CC_{50}), and anti-HIV activity evaluated by the inhibition assay for the cytopathic effects of HIV-1_{IIIB} (EC_{50}), using AZT as a positive control (EC_{50} = 0.0034 μg/mL and CC_{50} >200 μg/mL).[25] Compounds **1**–**10** showed weak anti-HIV activity with EC_{50} ranging from 6.1 to 22.1 μg/mL, and compounds **1**, **7**–**10** exerted minimal cytotoxicity against C8166 cells (CC_{50} >200 μg/mL) (Table 2). Among them, compound **1** demonstrated the most potent anti-HIV activity with an EC_{50} value of 6.1 μg/mL, a CC_{50} value of more than 200 μg/mL, and therefore a therapeutic index of greater than 32.8.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (20762015 and 20862019) and Yunnan Provincial Natural Science Foundation (2007C198M).

Supplementary data

Supplementary data (^1H and ^{13}C NMR, ^1H–^1H COSY, HMQC, HMBC, NOESY, EIMS, HRESIMS, UV and IR spectra data of angustific acid A (**1**)) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.055.

References and notes

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18. The air-dried and powdered branches (5 Kg) were extracted with 70% aqueous Me_2CO (4 × 20 L) at room temperature and concentrated in vacuo to give a crude extract, which was partitioned between H_2O and EtOAc. The EtOAc-soluble portion (165 g) was subjected to CC chromatography over MCI gel CHP 20P (H_2O/CH_3OH 1:0, 3:7, 6:4, 8:2, 0:1) to afford five main fractions (I–V) based on TLC behavior. Fraction II was further subjected to CC on silica gel by normal phase MPLC using a gradient with $CHCl_3$-acetone (9:1 to 6:4) to give two main subfractions [IIa (207 mg) and IIb (157 mg)]. Each subfraction was finally separated on a Sephadex LH-20 gel column eluting with acetone to yield pure compounds **3** (30 mg) and **4** (21 mg). Fraction III was also further separated on silica gel by normal phase MPLC using a gradient mixtures of petroleum ether, EtOAc, and CH_3COOH to give four main subfractions [IIIa (152 mg), IIIb (282 mg), IIIc (157 mg) and IIId (163 mg)], each of which was followed by a Sephadex LH-20 gel column eluting with CH_3COCH_3 to yield compounds **9** (22 mg), **7** (38 mg), **1** (21 mg) and **2** (18 mg), respectively. The separation of fraction IV by silica gel column chromatography eluted with petroleum ether-acetone with increasing polarity yielded four main subfractions [IVa (87 mg), IVb (138 mg), IVc (212 mg) and IVd (663 mg)], each of which was further purified by a Sephadex LH-20 gel column eluting with CH_3OH to yield **8** (16 mg), **10** (24 mg), **5** (56 mg) and **6** (256 mg), respectively.
19. Angustific acid A (**1**): light yellow oil (CH_3COCH_3); $[\alpha]_D^{16.4}$ +22.6° (CH_3OH; *c*0.64); UV (CH_3OH) λ_{max} (log ε) 203.2 (3.76) nm; IR (film) ν_{max} 3582–3240, 3068, 3045, 2946, 2878, 1702, 1640, 1456, 1414, 1373, 1262, 1234, 1085, 1048, 993, 947, 891 cm^{-1}; EIMS *m*/*z* (rel. int) 465 $[M + 1]^+$ (8), 446 (6), 439 (5), 404 (4), 391 (4), 383 (15), 365 (25), 351 (9), 183 (20), 171 (37), 157 (75), 145 (100), 139 (35), 120 (43), 105 (54), 95 (61), 79 (33); HREIMS *m*/*z*: 464.2933 $[M]^+$ (calcd for $C_{30}H_{40}O_4$, 464.2927).
20. Angustific acid B (**2**): colorless solid (CH_3COCH_3); $[\alpha]_D^{16.4}$+29.7° (CH_3OH; *c*0.64); UV (CH_3OH) λ_{max} (log ε) 204.4 (3.78) nm; IR (film) ν_{max} 3582–3240, 3068, 2977, 2886, 1702, 1641, 1456, 1411, 1375, 1263, 1213, 1165, 1081, 1063, 993, 893 cm^{-1}; ESIMS *m*/*z* (rel. int) 539 $[M + Na]^+$ (8), 1056 $[2M + Na+1]^+$ (1); HREIMS *m*/*z*: 539.2987 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_7Na$, 539.2984).
21. Angustifodilactone A (**3**): colorless solid (CH_3COCH_3); $[\alpha]_D^{23.9}$+19.7° (CH_3OH; *c*0.24); UV (CH_3OH) λ_{max} (log ε) 253.0 (3.75), 206.2 (3.76) nm; CD (*c* = 0.01, CH_3OH) ($\Delta\varepsilon$) 238 (−6.9), 279 (+27.6); IR (film) ν_{max} 3418, 2984, 2942, 2881, 1703, 1656, 1609, 1424, 1385, 1371, 1338, 1294, 1245, 1120, 1095, 1030, 994, 951, 911, 858, 832, 755 cm^{-1}; EIMS *m*/*z* (rel. int) 530 $[M]^+$ (1), 512 $[M - H_2O]^+$ (2), 494 $[M - 2H_2O]^+$ (4), 475 $[M - 3H_2O-1]^+$ (4), 457 $[M - 4H_2O -1]^+$ (4), 387 (5), 367 (29), 349 (15), 341 (24), 323 (43), 308 (35), 299 (19), 281 (42), 263 (27), 217 (60), 159 (68), 145 (100), 135 (83), 109 (87), 91 (56); TOFMS *m*/*z* (rel. int) 1083 $[2M + Na]^+$, 553 $[M + Na]^+$; HRMS (ESI-TOF) *m*/*z*: 553.2788 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_8Na$, 553.2777).
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24. Angustifodilactone B (**4**): colorless solid (CH_3COCH_3); $[\alpha]_D^{25.6}$+24.8° (CH_3OH; *c*0.18); UV (CH_3OH) λ_{max} (log ε) 255.0 (4.08), 204.6 (5.16) nm; CD (*c* = 0.01, CH_3OH) ($\Delta\varepsilon$) 240 (−7.3), 281 (+29.6); IR (film) ν_{max} 3428, 2981, 2943, 2881, 1701, 1660, 1547, 1424, 1383, 1374, 1336, 1302, 1290, 1246, 1122, 1097, 1049, 1029, 912, 855, 829, 787 cm^{-1}; TOFMS *m*/*z* (rel. int) 1051 $[2M + Na]^+$, 537 $[M + Na]^+$; HRMS (ESI-TOF) *m*/*z*: 537.2825 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_7Na$, 537.2828).
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