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Velutabularins A–J, phragmalin-type limonoids with novel cyclic moiety from *Chukrasia tabularis* var. *velutina*

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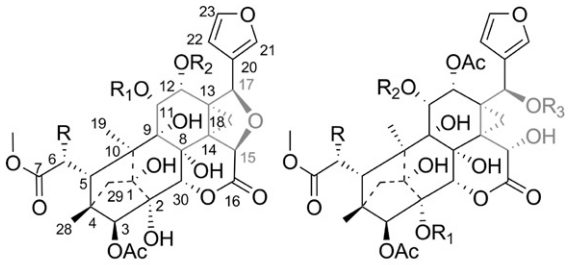
ABSTRACT

Velutabularins A–J, ten novel phragmalin-type limonoids, were isolated from the stem bark of *Chukrasia tabularis* var. *velutina*. In structures of **1**–**6**, the tetrahydrofuran ring from dehydration of OH-15 and OH-17, ring C and 13/14/18-cyclopropanyl moiety formed an unprecedented 8-oxatricyclo[4,3,1^{1,6}]decane. Compounds **7**–**10** are derivates of **1**–**6** opening the tetrahydrofuran ring. All of these compounds possess a novel C-16/C-30 δ-lactone ring, which were reported in phragmalins rarely. The structures of these novel compounds were elucidated based on extensive 1D and 2D spectroscopic analysis. The absolute configuration of **5** and **9** were determined by the calculated electronic circular dichroism (ECD) method. The anti-inflammatory activities of major compounds (**2**, **4**, **5**, **9**) were evaluated for inhibitory activity against lipopolysaccharide (LPS) induced nitric oxide (NO) production in macrophage (RAW264.7) cell line.

1. Introduction

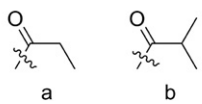
Phragmalin-type limonoids, found mainly in Swietenieae tribe of Meliaceae, are an important type of limonoids from Meliaceae.[1] Recent researches indicated that plants of *Chukrasia* were rich source of phragmalin-type limonoids and a series of phragmalins with attractive skeletons had been isolated in recent years, such as tabularisins with novel phragmalin skeleton incorporating a cyclopropanyl ring,[2] chuktabularins and chuktabrins with unprecedented 16-norphragmlin skeleton.[3] The structural diversity and potential biological significance of phragmalins[4] have prompted us to investigate the plants of genus *Chukrasia*.

Previously, we have reported six C-15-acyl phragmalin-type limonoids featuring a C-16/C-30 δ-lactone ring[5] and a series of 16-norphragmalin limonoids[6] from *Chukrasia tabularis* var. *velutina*.[7] As an ongoing investigation on the constituents of this plant, ten novel phragmalin-type limonoids with a 13/14/18-cyclopropanyl ring were isolated, which differ in carbon skeleton from those discovered in our previous research. In structures of **1**–**6**, the tetrahydrofuran ring from dehydration of OH-15 and OH-17, ring C and 13/14/18-cyclopropanyl moiety formed an unprecedented 8-oxatricyclo[4,3,1^{1,6}]decane in phragmalins. Compounds **7**–**10** were derivates of **1**–**6** opening the tetrahydrofuran ring. All of these compounds possess a novel C-16/C-30 δ-lactone ring, which encountered in phragmalins rarely. The structures of these novel compounds were elucidated on extensive 1D and 2D spectroscopic analyses. The absolute configuration of compounds **5** and **9** were determined by the calculated electronic circular dichroism (ECD) method, and those of others were proposed by correlating with them spectroscopically and biogenetically. The preliminary results of anti-inflammatory



	R	R_1	R_2
1	H	a	Ac
2	H	b	Ac
3	OAc	Ac	b
4	OAc	H	b
5	OAc	b	Ac
6	OAc	Ac	Ac

	R	R_1	R_2	R_3
7	OAc	H	Ac	b
8	OAc	H	b	Ac
9	OAc	b	Ac	Ac
10	H	H	Ac	b



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activities of major compounds (**2**, **4**, **5**, **9**), evaluating for inhibitory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in macrophage (RAW264.7) cell line, indicated that tested compounds exhibited moderate activity with IC_{50} value of 19.01, 10.09, 27.08, and 46.34 μM, respectively. These data indicated that the presence of tetrahydrofuran ring and free exocentric hydroxyl group maybe important to their anti-inflammatory activity. The cytotoxic activity test suggested that these compounds were inactive (IC_{50}>100 μM). Herein, the isolation, structural elucidation as well as the anti-inflammatory activity of these novel compounds were reported.

2. Results and discussion

Velutabularin A (**1**), white amorphous powder, has a molecular formula $C_{34}H_{40}O_{16}$ as established by the HRESIMS ion at *m/z* 727.2236 $[M+Na]^+$ (calcd: $C_{34}H_{40}O_{16}Na$, 727.2209), indicating 15 degrees of unsaturation. The data from decoupling and subsequent 1D and 2D NMR studies of **1** (^1H, ^{13}C, HMBC, HSQC, and ROESY) revealed that 8 of 34 carbon signals in ^{13}C NMR spectra were signals of two acetyl, one propionyl, and one methoxyl groups. Some characteristic moieties of phragmalin-type limonoid were also presented, such as a β-substituted furanyl ring (δ_H 6.54, 7.40, and 7.40; δ_C 121.0, 110.2, 143.1, and 142.0), characteristic signals of 4,29,1-ring bridge [δ_H 1.75 and 2.06 (d, *J*=11.6 Hz); δ_C 40.3], and the methyl esterified C-6-C-7 appendage. Aforementioned information suggested that **1** was a phragmalin-type limonoid with 26 carbons in skeleton.[8]

In ^1H NMR spectra of **1**, a pair of geminal doublets at δ_H 0.64 and 1.94 (d, *J*=4.5 Hz) was assigned to characteristic proton signals of a cyclopropane, which exhibited strong HMBC correlations (Fig. 1) with carbons of C-13 (δ_C 38.4), C-14 (δ_C 36.8), C-12 (δ_C 64.5), C-15 (δ_C 75.9), C-17 (δ_C 77.9), and C-8 (δ_C 70.7). These evidences indicated the presence of a 13/14/18-cyclopropanyl ring in **1**.[2] A singlet proton signal at δ_H 5.05 was assignable to H-17 from its HMBC correlations with C-20 (δ_C 124.1), C-12 (δ_C 64.5), and C-13 (δ_C 38.4), which showed significant difference from tabularin A (H-17, δ_H 6.42)[2a], a phragmalin-type limonoid with a 13/14/18-cyclopropanyl ring and C-16/C-17 δ-lactone ring isolated from *C. tabularis*. In addition, both carbon and proton signals of C-15 and C-17 were found to be mutually correlated in HMBC spectrum (Fig. 1). These evidences suggested the opening of the usual C-16/C-17 lactone ring and an ether linkage between C-17 and C-15, producing a tetrahydrofuran ring of C-13/C-14/C-15/C-17, which fused with ring C and 13/14/18-cyclopropanyl moiety to form an unprecedented 8-oxatricyclo [4,3,$1^{1,6}$]decane.

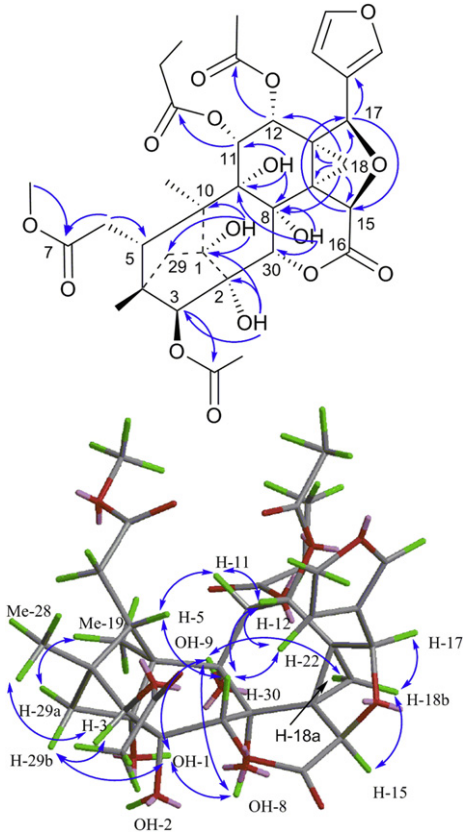

Fig. 1. Important HMBC and ROESY correlations of **1**.

In the light of the above analyses and degree of unsaturation, an additional lactone ring through COOH-16 was required. However, the location could not be determined directly by the HMBC spectrum since no valuable correlations observed for C-16 (δ_C 168.7). Fortunately, this uncertainty can be resolved by the assignments of relevant hydroxyl proton signals. HMBC correlations from H-3 (δ_H 4.71, s) and H-12 [δ_H 5.23 d (5.5)] to carbonyl signals of acetyl at δ_C 168.9 and δ_C 170.1, H-11 [δ_H 4.63, d (5.5)] to carbonyl signal of propionyl at δ_C 171.6 indicated that OH-3 and OH-12 were acetylated and OH-11 was propionylated. Four proton signals at δ_H 4.52, 5.30, 6.57, and 6.97 should be those of four remaining hydroxyl groups since no HSQC correlations observed for them. HMBC correlations, from signal at δ_H 4.52 to C-8 (δ_C 70.7), C-9 (δ_C 77.9), and C-11 (δ_C 73.8); δ_H 5.30 to C-2 (δ_C 74.6) and C-1 (δ_C 82.9); δ_H 6.57 to C-1, C-2, and C-29 (δ_C 40.4); δ_H 6.97 to C-8, C-9, and C-30 (δ_C 75.8), helped us to assign them to be proton signals of OH-9, OH-2, OH-1, and OH-8, respectively. Thus, the sole possible location of the lactone ring was confined to C-16/C-30 δ-lactone ring, and the plane structure of **1** was determined finally.

The relative stereochemistry of **1** was elucidated by the ROESY spectrum (Fig. 1). Strong cross peaks from the H-18b [δ_H 0.64 d (4.0)] to H-17 (δ_H 5.05) and H-15 (δ_H 4.51) indicated that 13/14/18-cyclopropanyl ring, H-17, and H-15 were cofacial and adopted an α-orientation.[2a] ROESY correlations of H-5 (δ_H 2.34) with H-11 [δ_H 4.63, d (5.5)] and H-30 (δ_H 4.62), H-12 [δ_H 5.23 d (5.5)] with H-11 and H-30 elucidated these protons to be β-orientated. The α-orientation of H-3, Me-19, H-29, OH-1, OH-8, and OH-9 were determined by the correlations of H-29b with H-3 and OH-1, H-29a with Me-19, OH-1 with OH-8 and OH-9. Thus, the structure of compound **1** was demonstrated as depicted.

Velutabularin B (**2**) was isolated as white amorphous powder. Its molecular formula was established as $C_{35}H_{42}O_{16}$ by HRESIMS ion at *m/z* 741.2388 $[M+Na]^+$ (calcd: $C_{35}H_{42}O_{16}Na$, 741.2365). The close similarity of its ^1H and ^{13}C NMR data (Tables 1 and 2) to those of **1** indicated that **2** was a derivative of **1** with the propionyl replaced by an isobutyryl group. Proton signal at δ_H 4.86 (d, 5.5) exhibited HMBC correlations to C-10 (δ_C 52.9), C-13 (δ_C 39.1), and a carbonyl signal of isobutyryl at δ_C 174.6 indicated that the isobutyryl group esterificated at OH-11. The relative configuration of **2** was determined to be the same as that of **1** using ROESY spectra. Thus, the structure of **2** was determined as 11-*O*-isobutyryl derivative of 11-*O*-depropionylated **1**.

Velutabularin C (**3**), white amorphous powder, was found to possess a molecular formula of $C_{37}H_{44}O_{18}$ from its HRESIMS ion at *m/z* 799.2434 $[M+Na]^+$ (calcd: $C_{37}H_{44}O_{18}Na$, 799.2420). The resemblance of its NMR features to those of **1** and the key HMBC correlations from H-15 (δ_H 4.52) to C-17 (δ_C 78.4), and H-17 (δ_H 5.06) to C-15 (δ_C 76.3) indicated that **3** was also a phragmalin-type limonoid with an unprecedented 8-oxatricyclo[4,3,$1^{1,6}$]decane and C-16/C-30 δ-lactone ring like **1**. In HMBC spectrum of **3**, a single proton signal at δ_H 5.49 was assignable to H-6 from its correlations observed with the quaternary carbon signals at δ_C 45.1 (C-5), 170.0 (C-7), and 170.1

Table 1
^{1}H NMR data of compounds **1**–**6**

Position	**1**[a]	**2**[b]	**3**[c]	**4**[a]	**5**[b]	**6**[a]
3	4.71, s	4.77, s	4.68, s	4.67, s	4.73, s	4.67, s
5	2.34, br s	2.50, m*	2.59, br s	2.59, br s	2.69, br s	2.59, br s
6a	2.33, m*	2.50, m*	5.49, br s	6.36, br s	5.56, br s	5.48, br s
6b	2.26, m*	2.24, dd (18.0,11.5)				
11	4.63, d (5.5)	4.86, d (5.5)	4.44, d (6.5)	3.64, d (6.0)	4.51, d (6.0)	4.43, d (6.0)
12	5.23, d (5.5)	5.44, d (5.5)	5.42, d (6.5)	4.57, d (6.0)	5.76, d (6.0)	5.45, d (6.0)
15	4.51, s	4.63, s	4.52, s	4.52, s	4.64, s	4.52, s
17	5.05, s	5.25, s	5.06, s	5.06, s	5.28, s	5.06, s
18a	1.94, d (4.0)	1.97, d (4.0)	1.91, d (4.0)	1.82, d (4.0)	1.84, d (5.0)	1.91, d (4.0)
18b	0.64, d (4.0)	0.85, d (4.0)	0.60, d (4.0)	0.54, d (4.0)	0.80, d (5.0)	0.63, d (4.0)
19	1.05, s, 3H	1.19, s, 3H	1.12, s, 3H	1.12, s, 3H	1.28, s, 3H	1.10, s, 3H
21	7.57, br s	7.64, br s	7.64, br s	7.70, br s	7.50, br s	7.58, br s
22	6.19, d (1.0)	6.54, d (1.0)	6.16, br s	6.12, d (1.0)	6.47, br s	6.18, br s
23	7.49, t-like (1.5)	7.49, t-like (1.5)	7.86, br s	7.90, t-like (1.5)	7.50, br s	7.82, t-like (1.5)
28	0.66, s, 3H	0.81, s, 3H	0.86, s, 3H	0.88, s, 3H	0.99, s, 3H	0.85, s, 3H
29a	1.97, d (11.0)	2.09, d (11.0)	2.10, d (11.0)	2.10, d (11.0)	2.33, d (11.0)	2.09, d (10.5)
29b	1.50, d (11.0)	1.63, d (11.0)	1.64,,d (11.0)	1.57, d (11.0)	1.72, d (11.0)	1.64, d (10.5)
30	4.62, s	4.61, s	4.49, s	4.51, s	4.44, s	4.47, s
OH-1	6.57, s		6.59, s			6.57, s
OH-2	5.30, s		5.43, s			5.32, s
OH-8	6.97, s		7.13, s			7.09, s
OH-9	4.52, s		4.91, s			4.81, s
OCH_3-7	3.60, s, 3H	3.60, s, 3H	3.55, s, 3H	3.60, s, 3H	3.63, s, 3H	3.54, s, 3H
OAc-3	2.00, s, 3H	2.16, s, 3H	2.17, s, 3H	2.11, s, 3H	2.13,,s, 3H	2.06,,s, 3H
OAc-6			2.10, s, 3H	2.11, s, 3H	2.19, s, 3H	2.15, s, 3H
R-11	$OCOCH_2CH_3$-11	$OCOCH(CH3)_2$-11	OAc-11		$OCOCH(CH3)_2$-11	OAc-11
	2.15, 2.23, m, 2H*	2.50, m*	1.98, s, 3H		2.27, m*	1.98, s, 3H
	0.92, t (7.5), 3H	1.08, d (7.0), 3H			1.20, d (7.0), 3H)	
		1.19, d (7.0), 3H			1.03, d (7.0), 3H)	
R-12	OAc-12	OAc-12	$OCOCH(CH_3)_2$-12	$OCOCH(CH3)_2$-12	OAc-12	OAc-12
	2.10, s, 3H	2.05, s, 3H	2.53 m*	2.61, m*	2.08, s, 3H	2.02, s, 3H
			1.11, d (7.0), 3H	1.14, d (7.0), 3H)		
			1.09, d (7.0), 3H	1.07, d (7.0), 3H)		

* Signal pattern unclear due to overlapping.
[a] Recorded at 500 MHz in DMSO-d_6.
[b] Recorded at 500 MHz in $CDCl_3$.
[c] Recorded at 600 MHz in DMSO-d_6.

(OAc-6), which suggested that C-6 was acetoxylated. On the basis of the corresponding HMBC correlations, δ_H 4.68 (H-3) to δ_C 169.2 (OAc-3); δ_H 4.54 (d, 6.5, H-11) to δ_C 168.9 (OAc-11); δ_H 5.42 (d, 6.5, H-12) to δ_C 176.5 (OiBuy-12), two acetoxyls and an isobutoxyryl was assignable to C-3 (δ_C 86.7), C-11 (δ_C 76.1), and C-12 (δ_C 64.3), respectively. Strong cross peaks in the NOESY experiment indicated a β-orientation of H-5, H-11, H-12, H-17, H-30, and Me-28, and α-orientation of H-3, H-14, Me-19, and H-29 the same as those of compound **1**. The NOESY correlation of H-6 with H-5, H-11, and H-12, and agreement of the ^{1}H and ^{13}C NMR spectroscopic data at left side chain of **3** with those of tabularisins established H-6 as being in a β-orientation, which was determined by single-crystal X-ray diffraction experiment.[2] Thus, the structure of **3** was established as shown.

Velutabularin D (**4**) was isolated as white amorphous powder with a molecular formula of $C_{35}H_{42}O_{17}$ by its HRESIMS ion at m/z 757.2323 (calcd for $C_{35}H_{42}O_{17}Na$: 757.2314). The similarity of its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) and the difference of its molecular formula from that of **3** indicated that **4** was a deacetyl derivative of **3**. On the basis of corresponding HMBC correlations, the acetyls and isobutyryl was assigned at OH-3, OH-6, and OH-12, respectively. The relative configurations of compound **4** including asymmetric C-6 were established using the NOESY spectra to be the same as those of **3**. Thus, the structure of **4** was established as 11-*O*-deacetyl derivative of **3**.

The molecular formula of velutabularin E (**5**) was determined to be $C_{37}H_{44}O_{18}$ based on its HRESIMS quasimolecular ion at m/z 799.2409 $[M+Na]^+$. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) and the information obtained from the subsequent 2D NMR studies indicated that **5** possessed the same skeleton as **3**. The same molecular formula indicated that **3** and **5** were isomer, and the difference was the location of substituted ester groups. Specification of the sites of ester linkages was obtained by HMBC experiments. HMBC correlation from H-11 (δ_H 4.51) to the carbonyl signal of the isobutryl at δ_C 174.9 revealed that the OH-11 was isobutrylated in **5**. Three acetyl groups esterificated at OH-3, OH-6, and OH-12 according to their corresponding HMBC correlations. The relative configurations of asymmetric carbons including C-6 were established using the NOESY spectra to be the same as those of **3**. Thus, the structure of **5** was established as 11-*O*-isobutyryl, 12-*O*-acetyl derivative of 11-*O*-deacetylated, 12-*O*-deisobutyrylated **3**.

To determine its absolute configuration, the CD spectrum of **5** was measured in CH_3CN, which revealed a positive Cotton effect at λ_{max} 222 nm ($\Delta\epsilon$+1.29). We calculated the electronic circular dichroism (ECD) by time-dependent density functional theory (TDDFT),[9] and compared the result with experimental CD data of **5**. The conformational analysis was performed by means of the semiempirical PM3 method, as implemented in the program package Q-Chem, starting from preoptimized geometries generated by the MM2 force field in Chem 3D software overlaid with key correlations observed in the ROESY spectrum. The corresponding minimum geometries found were further optimized by DFT calculations at the B3LYP/6-31G(d) level. The calculated ECD of **5** (Fig. 2) matches the experimental result, allowing the assignment of the absolute configuration of **5** as depicted.

Velutabularin F (**6**), white amorphous powder, its molecular formula was determined to be $C_{35}H_{40}O_{18}$ based on the quasimolecular ion at m/z 771.2090 $[M+Na]^+$ in HRESIMS. The similarity of its ^{1}H and ^{13}C NMR spectra and the information from the HRESIMS indicated that **6** and **5** were homologous, and the difference was

Table 2
^{13}C NMR data of compounds **1**–**6**

Position	**1**[a]	**2**[b]	**3**[c]	**4**[a]	**5**[b]	**6**[a]
1	82.9	83.4	83.7	82.7	83.6	83.1
2	74.6	74.6	75.0	74.7	74.6	74.8
3	86.0	86.2	86.7	86.4	86.7	86.7
4	43.8	43.8	43.9	43.2	43.2	43.4
5	39.5	40.3	45.1	44.7	45.3	44.5
6	32.9	33.7	71.4	71.4	71.2	70.7
7	172.2	171.9	170.0	170.7	169.4	169.4
8	70.7	71.6	71.2	71.1	71.6	70.8
9	77.9	77.9	79.1	78.4	78.7	78.5
10	52.7	52.9	54.7	54.0	54.4	54.1
11	73.8	72.9	76.1	72.2	75.0	75.3
12	64.5	65.8	64.3	67.6	64.5	64.0
13	38.4	39.1	39.8	39.3	40.8	39.4
14	36.8	37.1	37.2	36.4	37.3	36.8
15	75.9	76.9	76.3	75.9	76.7	75.9
16	168.7	167.9	169.2	169.0	168.2	168.6
17	77.9	79.6	78.4	77.5	79.3	77.9
18	17.7	17.9	18.0	17.4	17.9	17.8
19	15.5	15.1	15.5	14.7	14.6	14.9
20	124.1	124.2	124.8	124.4	124.8	124.3
21	140.9	140.6	141.7	141.4	140.7	141.0
22	107.6	108.4	107.9	107.4	108.7	107.5
23	144.7	144.4	145.3	144.6	144.1	144.6
28	14.8	14.8	16.3	15.7	15.9	15.8
29	40.4	42.0	41.9	41.6	42.7	41.4
30	75.8	75.1	76.0	75.9	74.6	75.4
OCH_3-7	51.8	51.8	52.9	52.3	52.6	52.3
OAc-3	168.9	170.4	169.2	168.8	169.8	168.6
	20.5	20.4	21.1	20.4	20.3	20.3
OAc-6			170.1	169.1	169.7	169.5
			20.9	20.8	21.0	20.7
OAc-11			168.9			168.4
			21.1			20.5
$OCOCH_2$	171.6					
CH_3-11	27.0					
	8.6					
OCOCH		174.6			174.9	
$(CH3)_2$-11		34.4			34.1	
		19.1			20.3	
		18.0			17.6	
OAc-12	170.1	169.9			170.2	170.3
	20.5	20.8			20.9	20.5
OCOCH			176.5	176.4		
$(CH3)_2$-12			33.7	32.9		
			19.3	19.1		
			19.1	18.3		

[a] Recorded at 125 MHz in DMSO-d_6.
[b] Recorded at 125 MHz in $CDCl_3$.
[c] Recorded at 150 MHz in DMSO-d_6.

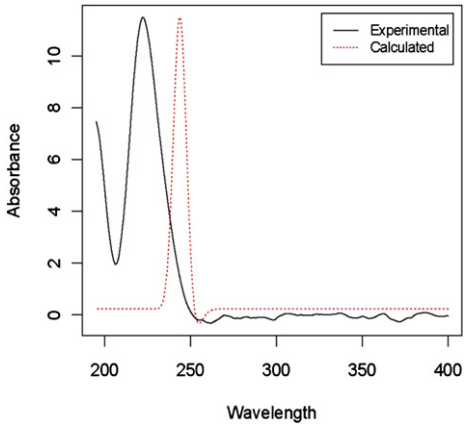

Fig. 2. ECD spectra of **5**: experimental in plain, calculated in dash.

that the isobutyryl in **5** replaced by an acetyl in **6**. The HMBC correlations, from H-3 (δ_H 4.67), H-6 (δ_H 5.48), H-11 (δ_H 4.43), and H-12 (δ_H 5.45) to carbonyl signals at δ_C 168.6, δ_C 169.5, δ_C 168.4, and δ_C 170.3, conformed that four acetyl groups attached at OH-3, OH-6, OH-11, and OH-12, respectively. Their relative configurations were established using the NOESY spectra to be the same as that of **3**. Thus, the structure of **6** was established as 11-*O*-acetyl derivative of 11-*O*-deisobutyrylated **5**.

Velutabularin G (**7**), white amorphous powder, has a molecular formula of $C_{39}H_{48}O_{20}$, as established by the HRESIMS ion at *m*/*z* 859.2652 (calcd for $C_{39}H_{48}O_{20}Na$: 859.2631). The data from 1D and 2D NMR studies of **7** (^{1}H, ^{13}C, HMBC, HSQC, and NOESY) revealed that **7** was also a phragmalin-type limonoid with a 13/14/18-cyclopropanyl ring.

Carefully inspection of the ^{1}H NMR data of **7** and **1** revealed that the proton signals of 13/14/18-cyclopropanyl ring downfield shifted from δ_H 0.64 and 1.94 (d, *J*=4.5 Hz) in **1** to δ_H 1.15 and 1.84 (d, *J*=5.0 Hz) in **7**. A characteristic singlet proton signal at δ_H 5.97 (H-17) showed HMBC correlations (Fig. 3) with C-12 (δ_C 64.1), C-13 (δ_C 31.7), C-14 (δ_C 37.7), C-20 (δ_C 123.3), C-21 (δ_C 140.1), C-22 (δ_C 109.2), and a carbonyl of isobutyryl (δ_C 174.6). Above evidences suggested the opening of the ether linkage between C-15 and C-17 in **1** and isobutyrylation of OH-17. HMBC correlations between proton signals of carbon framework and ester carbonyl carbons, e.g., δ_H 4.60 (H-3) to δ_C 169.1 (OAc-3); δ_H 5.49 (H-6) to δ_C 169.3 (OAc-6); δ_H 4.61 (H-11) to δ_C 168.5 (OAc-11); δ_H 6.26 (H-12) to δ_C 169.0 (OAc-12), indicated that OH-3, OH-6, OH-11, and OH-12 were acetylated. Similar to **1**, the location of lactone ring through COOH-16 could not be resolved directly by HMBC spectra of **7**. Likewise, the presence of C-16/C-30 δ-lactone ring in **7** was confirmed after assignment of proton signals of OH-1 (δ_H 6.29), OH-2 (δ_H 4.74), OH-8 (δ_H 6.19), OH-9 (δ_H 4.74), and OH-15 (δ_H 6.11) through comprehensive analyses of the HMBC and HSQC spectra.

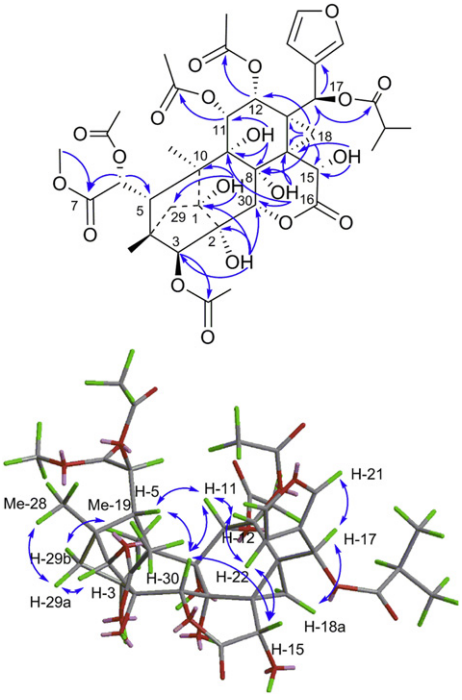

Fig. 3. Key HMBC and NOESY correlations of **7**.

An NOESY experiment (Fig. 3) of **7** indicated that all the asymmetric carbons had the same relative stereochemistry as those of **1**, and the only difference was the orientation of OH-15. Strong cross peaks from H-15 (δ_H 4.40) to H-30 (δ_H 4.45) and H-22 (δ_H 4.65)

suggested the α-orientation of OH-15, on the contrary to β-OH in **1**. The NOESY correlation of H-6 with H-5, H-11, H-12, and Me-19, and agreement of the ^{1}H and ^{13}C NMR spectroscopic data at left side chain of **7** with those of **3** and tabularisins established H-6 as being in a β-orientation, which was determined by single-crystal X-ray diffraction experiment.[2] Thus, the structure of **7** was demonstrated as depicted.

Velutabularin H (**8**) was isolated as white amorphous powder. Its molecular formula was established as $C_{39}H_{48}O_{20}$ by its HRESIMS ion at *m*/*z* 859.2642 $[M+Na]^+$ (calcd: $C_{39}H_{48}O_{20}Na$: 859.2631). The similarity of its ^{1}H and ^{13}C NMR spectral data to those of **7** (Table 3) and the HRESIMS data indicated that **8** was an esterfunction positional isomer of **7**. HMBC correlation from H-11 (δ_H 4.62) to the carbonyl signal of the isobutryl at δ_C 173.4 revealed that the OH-11 was isobutrylated in **8**. Other key HMBC correlation between proton signals of basic skeleton and carbonyl carbons of esterfunction indicated that OH-3, OH-6, OH-12, and OH-17 were acetylated. The relative configurations of asymmetric carbons in **8** including C-6 were established using the NOESY spectra to be the same as those of **7**. Thus, the structure of **8** was determined to be 11-*O*-isobutyryl and 17-*O*-acetylated derivative of 11-*O*-deacetylated and 17-*O*-deisobutyrylated **7**.

Velutabularin I (**9**) exhibited quasimolecular ion at *m*/*z* 901.2718 $[M+Na]^+$ in its HRESIMS, indicating the molecular formula of $C_{41}H_{50}O_{21}$. Information from 1D and 2D NMR data and the molecular formula indicated that compound **9** was an acetyl derivative of **8**. The key HMBC correlations from proton signals to ester carbonyl carbons revealed that five acetyl groups were attached at OH-3, OH-6, OH-11, OH-12, and OH-15. No correlation was observed for the carbonyl carbon of isobutyryl, which indicated that the acylation was at a quaternary carbon. Compared with **8**, the significant downfield shifted signals of C-2 ($\Delta\delta_C$ 6.2) suggested that OH-2 was esterificated and that was the isobutyryl group. Comprehensive analysis of its HMBC and HSQC spectra allowed the assignments of

Table 3
^{1}H NMR and ^{13}C NMR data of **7**–**10**

No.	**7**[a]		**8**[a]		**9**[b]		**10**[a]	
	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C
1		83.9		83.7		84.2		83.8
2		73.6		73.5		79.8		73.4
3	4.60, s	85.2	4.57, s	85.1	4.99, s	82.9	4.62, s	84.9
4		43.6		43.6		44.7		44.1
5	2.60, br s	44.2	2.64, br s	44.2	2.72, br s	45.0	2.26, m[c]	39.2
6	5.49, br s	71.2	5.54, br s	71.3	5.64, d (1.5)	71.2	2.36, 2.26, m[c]	33.3
7		169.6		169.9		170.1		172.8
8		74.8		74.6		76.0		74.6
9		76.5		76.7		77.1		76.1
10		53.5		53.3		54.7		51.9
11	4.61, d (5.0)	73.8	4.62, d (5.4)	74.4	4.94, d (5.5)	73.5	4.66, d (5.4)	73.6
12	6.26, d (5.0)	64.1	6.38, d (5.4)	63.6	6.63, d (5.5)	65.6	6.19, d (5.4)	64.3
13		31.7		32.1		32.6		31.4
14		37.7		37.3		38.5		37.3
15	4.40, d (5.5)	63.2	4.30, br s	63.1	4.35, br s	63.0	4.33, br s	63.2
16		172.3		172.1		172.3		172.3
17	5.97, s	69.9	5.98, s	69.5	5.91, s	69.9	5.96, s	69.9
18a	1.84, d (5.0)	13.4	1.80, d (5.0)	13.5	1.66, d (6.0)	13.8	1.83, d (5.0)	13.5
18b	1.15, d (5.0)		1.21, d (5.0)		1.56, d (6.0)		1.18, d (5.0)	
19	1.02, s, 3H	17.0	1.05, s, 3H	16.7	1.16, s, 3H	17.4	0.98, s, 3H	17.6
20		123.3		123.2		123.0		123.3
21	7.71, br s	140.1	7.71, br s	140.5	7.78, br s	141.1	7.71, br s	140.2
22	6.65, d (1.0)	109.2	6.76, d (1.0)	109.2	6.68, d (1.0)	109.7	6.64, d (1.0)	109.2
23	7.67, t-like (1.5)	143.7	7.74, t-like (1.5)	143.9	7.42, t-like (1.5)	143.9	7.67, t-like (1.5)	143.8
28	0.75, s, 3H	16.5	0.77, s, 3H	16.5	0.90, s, 3H	16.8	0.61, s, 3H	16.1
29a	1.97, d (11.0)	40.8	1.97, d (11.0)	40.9	2.26, d (11.0)	41.7	1.76, d (11.0)	39.0
29b	1.76, d (11.0)		1.74, d (11.0)		1.81, d (11.0)		1.65, d (11.0)	
30	4.45, s	73.7	4.42, s	73.6	4.78, s	73.7	4.46, s	73.6
OH-1	6.29, s				4.35, s		6.46, s	
OH-2	4.74, s						4.68, s	
OH-8	6.19, s				4.43, s		6.08, s	
OH-9	4.74, s				3.36, s		4.67, s	
OH-15	6.11, d (5.5)				3.44, br s		6.04, br s	
OMe-7	3.55, s, 3H	52.0	3.54, s, 3H	52.3	3.66, s, 3H	52.7	3.50, s, 3H	50.1
OAc-3		169.1		169.0		168.1		169.3
	1.96, s, 3H	20.1	1.97, s, 3H	20.3	2.04, s, 3H	20.3	1.99, s, 3H	20.3
OAc-6		169.3		169.3		169.7		
	2.14, s, 3H	20.8	2.14, s, 3H	20.7	2.18, s, 3H	22.1		
11-OAc		168.9				169.2		168.5
	1.77, s, 3H	21.0			1.98, s, 3H	21.5	1.72, s, 3H	20.7
12-OAc		169.0		169.3		168.9		169.9
	2.06, s, 3H	21.2	2.03, s, 3H	21.7	2.17, s, 3H	21.1	2.03, s, 3H	19.6
17-OAc				168.8		169.1		
			1.88, s, 3H	20.5	1.93, s, 3H	20.8		
R	$OCOCH(CH3)_2$-17		$OCOCH(CH3)_2$-11		$OCOCH(CH3)_2$-2		$OCOCH(CH3)_2$-17	
		174.6		173.4		176.4		174.6
	2.37 m	33.3	2.28 m	33.7	2.28 m	34.0	2.36 m	33.3
	1.02, d (7.0), 3H	18.5	1.10, d (7.0), 3H	18.3	1.27, d (7.0), 3H	18.9	1.01, d (7.0), 3H	18.3
	1.01, d (7.0), 3H	18.4	1.03, d (7.0), 3H	17.5	1.21, d (7.0), 3H	18.7	1.00, d (7.0), 3H	18.4

[a] Recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C) in DMSO-d_6.
[b] Recorded at 500 MHz (^{1}H) and 150 MHz (^{13}C) in $CDCl_3$.
[c] Signal pattern unclear due to overlapping.

the proton signals of OH-1 (δ_H 6.29), OH-2 (δ_H 4.74), OH-8 (δ_H 6.19), OH-9 (δ_H 4.74), and OH-15 (δ_H 6.11), consistent with the above deduction. The key NOESY correlations indicated the relative configurations of **9** including asymmetric C-6 to be the same as those of **7**. Thus, the structure of **9** was demonstrated as 2-*O*-isobutyryl and 11-*O*-acetyl derivative of 11-*O*-deisobutyrylated **8**. The absolute configuration of **9** was determined as depicted by the calculated electronic circular dichroism (ECD) method as compound **5**, which matched the experimental result very well (Fig. 4).

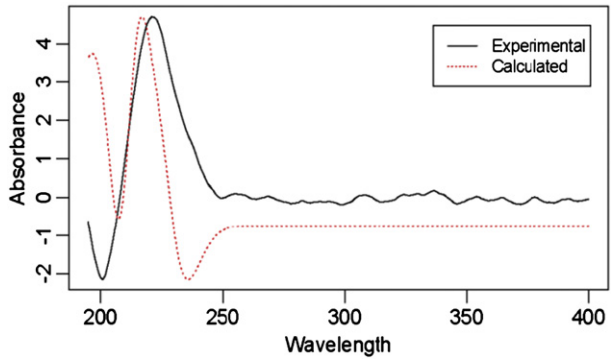

Fig. 4. ECD spectra of **9**: experimental in plain, calculated in dash.

Velutabularin J (**10**), a white amorphous powder, was found to possess a molecular formula of $C_{39}H_{48}O_{20}$ from the HRESIMS ion at *m/z* 801.2591 $[M+Na]^+$ (calcd: $C_{37}H_{46}O_{18}Na$, 801.2576). The ^1H and ^{13}C NMR data and key HMBC correlations indicated that **10** was a deacetoxyl derivate of **7**. In the HMBC spectrum of **10**, signal of C-7 (δ_C 172.8) showed strong correlations with a methoxyl (δ_H 3.50, 3H) and methylene proton signals (δ_H 2.26 and 2.36), which indicated that C-6 in **10** was not oxygenated. Key HMBC correlations revealed the same esterification sites in **10** as those of **7**, the acetylation of OH-3, OH-11, and OH-12, and the isobutyrylation of OH-17. Thus, the structure of **10** was determined as 6-deacetoxyl derivate of **7**.

The proposed biosynthetic pathway of these novel phragmalin-type limonoids was described as showing in Supplementary data.

The cytotoxic activity and anti-inflammatory activities of major compounds (**2**, **4**, **5**, **9**) were tested in this research. Cytotoxic activity against five human cancer cell lines, MCF-7 (human breast cancer), Hela (human cervical cancer), SGC-7901 and BGC-823 (human gastric cancer), HepG2 (human liver cancer), were tested by an MTT assay, whose result showed that all samples were non-cytotoxic (IC_{50}>100 μM). Subsequently, the anti-inflammatory activity of them were evaluated for inhibitory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in macrophage (RAW264.7) cell line, and dexamethasone was used as positive control substance with IC_{50} value of 0.06 μM. The preliminary results indicated that tested compounds (**2**, **4**, **5**, **9**) exhibited moderate inhibitory activity with IC_{50} value of 19.01, 10.09, 27.08, and 46.34 μM, respectively. These data illuminated that compounds with tetrahydrofuran ring (**2**, **4**, **5**) was more active than compound without it (**9**) and compound (**4**) with free exocentric hydroxyl group (OH-11) more active than those without it (**2**, **5**), which indicated that the presence of tetrahydrofuran ring and free exocentric hydroxyl group maybe important to their anti-inflammatory activity.

3. Experimental section

3.1. General experimental procedures

Optical rotations were measured with a *JASCO* P-1020 polarimeter. CD spectra were obtained on a *JASCO* 810 spectropolarimeter. IR (KBr-disks) spectra were recorded by Bruker Tensor 27 spectrometer. NMR spectra were recorded on Bruker ACF-500 or 600 NMR instrument (^1H: 500 or 600 MHz, ^{13}C: 125 or 150 MHz) with TMS as internal standard. Mass spectra were obtained on an MS Agilent 1100 Series LC/MSD iron-trap mass spectrometer (ESIMS) and a Mariner ESITOF spectrometer (HRESIMS), respectively. All solvents used were of analytical grade (Jiangsu Hanbang Science and Technology. Co., Ltd). Silica gel (Qingdao Haiyang Chemical Co., Ltd), Sephadex LH-20 (Pharmacia), and RP-C_{18} (40–63 μm, FuJi) were used for column chromatography. Preparative HPLC was carried out using Agilent 1100 Series with Shim-park RP-C_{18} column (20×200 mm) and a 1100 Series Multiple Wavelength detector.

3.2. Plant material

The air-dried stem bark of *C. tabularis* var. *velutina* was collected from Xishuangbanna, Yunnan Province, People's Republic of China, in March 2007, and was authenticated by Professor Mian Zhang of Research Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (No. 2006-MML) had been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

3.3. Extraction and isolation

The air-dried stem bark (10 kg) was extracted by refluxing with 95% ethanol three times. The EtOH extract was concentrated under reduced pressure (2000 g) and then extracted with $CHCl_3$ to give a chloroform extract (300 g). The oily chloroform extract was dissolved in 2 L 50% MeOH and H_2O and then extracted with petroleum ether. After removal of the fatty components, 210 g of extract was obtained, which was subjected to passage over a silica gel column eluted with $CHCl_3$–MeOH in a gradient (1:0 to 1:2), to obtain eight fractions (Fr. A–H) according to TLC monitor. Fr. E (20 g) was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH–H_2O (4:6 to 7:3) to give six sub-fractions (Fr. E1–E6). Fr. E6 was chromatographed on a column of silica gel eluted successively with a gradient of petroleum ether–EtOAc (2:1 to 1:3) to give three sub-fractions (Fr. E6a–c). Fr. E6a was separated by preparative HPLC using CH_3CN–H_2O (42:58, 10 mL/min) as the mobile phase to give **9** (10 mg). Fr. F (13 g) was chromatographed on a column of silica gel eluted successively with a gradient of petroleum ether–EtOAc (1:1 to 1:4) to give four sub-fractions (Fr. F1–4). Fr. F2 was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH–H_2O (2:3 to 7:3) to give four sub-fractions (Fr. F2a–d). Fr. F2c was separated by preparative HPLC using CH_3OH–H_2O (56:44, 10 mL/min) as the mobile phase to give **4** (5 mg). Fr. F2d was separated on a column of Sephadex LH-20 eluted with MeOH to give three sub-fractions, and each one was separated by preparative HPLC using CH_3OH/H_2O (56:44, 10 mL/min) as the mobile phase to afford **3** (5 mg) and **7** (8 mg), **8** (4 mg), and **10** (5 mg). Fr. F3 was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH–H_2O (2:3 to 7:3) to give four sub-fractions (Fr. F3a–d). Fr. F3d was separated by preparative HPLC using CH_3OH–H_2O (52:48, 10 mL/min) as the mobile phase to give **2** (6 mg) and **5** (20 mg). Fr. F4 was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH–H_2O (2:3 to 7:3) to give four sub-fractions (Fr. F4a–d). Fr. F4d was separated by preparative HPLC using CH_3OH–H_2O (55:45, 10 mL/min) as the mobile phase to give **1** (10 mg) and **6** (6 mg).

Velutabularin A (**1**): white, amorphous powder; $[\alpha]_{D^{25}}$ −46 (*c* 0.14, CH_3OH); IR (KBr) ν_{max} 3453, 2986, 1748, 1638, 1382, 1246, 1075, 1032 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1 and Table 2; negative ESIMS *m/z*: 703.1$[M-H]^-$ (100); positive ESIMS *m/z*: 722.2 $[M+NH_4]^+$ (100); HRESIMS *m/z*: 727.2236 (calcd $C_{34}H_{40}O_{16}Na$, 727.2209).

Velutabularin B (**2**): white, amorphous powder; $[\alpha]_{D^{25}}$ −43 (*c* 0.10, CH_3OH); IR (KBr) ν_{max} 3460, 2977, 1747, 1639, 1386, 1246, 1145 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1 and Table 2; negative ESIMS *m/z*: 717.5$[M-H]^-$ (100); positive ESIMS *m/z*: 736.3 $[M+NH_4]^+$ (100); HRESIMS *m/z*: 741.2388 $[M+Na]^+$ (calcd for $C_{35}H_{42}O_{16}Na$: 741.2365).

Velutabularin C (**3**): white, amorphous powder; $[\alpha]_{D^{25}}$ −20 (*c* 0.12, CH_3OH); IR (KBr) ν_{max} 3463, 2975, 1755, 1638, 1377, 1267, 1222, 1066, 1036, 603 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1 and Table 2; negative ESIMS *m/z*: 775.3$[M-H]^-$ (100); positive ESIMS *m/z*: 794.3 $[M+NH_4]^+$ (100); HRESIMS *m/z*: 799.2434 (calcd $C_{37}H_{44}O_{18}Na$, 799.2420).

Velutabularin D (**4**): white, amorphous powder; $[\alpha]_{D^{25}}$ −37 (*c* 0.10, CH_3OH); IR (KBr) ν_{max} 3455, 2974, 1740, 1640, 1377, 1227, 1145, 1063 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1 and Table 2; negative ESIMS *m/z*: 733.2 $[M-H]^-$ (100); positive ESIMS *m/z*: 752.3 $[M+NH_4]^+$ (100); HRESIMS *m/z*: 757.2323 $[M+Na]^+$ (calcd for $C_{35}H_{42}O_{17}Na$: 757.2314).

Velutabularin E (**5**): white, amorphous powder; $[\alpha]_{D^{25}}$ −44 (*c* 0.15, CH_3OH); IR (KBr) ν_{max} 3428, 2974, 1750, 1636, 1375, 1225, 1035 cm^{-1}; CD (CH_3CN, $\Delta\epsilon$) 222 (+1.29) nm; ^1H NMR and ^{13}C NMR data, see Table 1 and Table 2; negative ESIMS *m/z*: 775.5$[M-H]^-$ (100); positive ESIMS *m/z*: 794.4 $[M+NH_4]^+$ (100); HRESIMS *m/z*: 799.2409 $[M+Na]^+$ (calcd for $C_{37}H_{44}O_{18}Na$: 799.2420).

Velutabularin F (**6**): white, amorphous powder; $[\alpha]_{D^{25}}$ −34 (*c* 0.08, CH_3OH); IR (KBr) ν_{max} 3452, 1752, 1640, 1377, 1224, 1066, 1036 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1 and Table 2; negative ESIMS *m/z*: 747.1 $[M-H]^-$ (100); positive ESIMS *m/z*: 766.2 $[M+NH_4]^+$ (100); HRESIMS *m/z*: 771.2090 $[M+Na]^+$ (calcd for $C_{35}H_{40}O_{18}Na$: 771.2107).

Velutabularin G (**7**). White, amorphous powder; $[\alpha]_{D^{25}}$ −4 (*c* 0.05, CH_3OH); IR (KBr) ν_{max} 3425, 2975, 1755, 1639, 1374, 1224, 1031 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 3; negative ESIMS *m/z*: 835.5 $[M-H]^-$ (100); positive ESIMS *m/z*: 854.3 $[M+NH_4]^+$ (100); HRESIMS *m/z*: 859.2652 $[M+Na]^+$ (calcd for $C_{39}H_{48}O_{20}Na$: 859.2631).

Velutabularin H (**8**): white, amorphous powder; $[\alpha]_{D^{25}}$ −14 (*c* 0.05, CH_3OH); IR (KBr) ν_{max} 3442, 2988, 1753, 1639, 1372, 1222, 1031 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 3; negative ESIMS *m/z*: 835.3 $[M-H]^-$ (100); positive ESIMS *m/z*: 854.3 $[M+NH_4]^+$ (100); HRESIMS *m/z*: 859.2642 $[M+Na]^+$ (calcd for $C_{39}H_{48}O_{20}Na$: 859.2631).

Velutabularin I (**9**): white, amorphous powder; $[\alpha]_{D^{25}}$ −17 (*c* 0.13, CH_3OH); IR (KBr) ν_{max} 3463, 2976, 1760, 1639, 1377, 1231, 1098, 1026 cm^{-1}; CD (CH_3CN, $\Delta\epsilon$) 201 (−0.28), 221 (+0.60) nm; ^1H NMR and ^{13}C NMR data, see Table 3; negative ESIMS *m/z*: 877.4 $[M-H]^-$ (100); positive ESIMS *m/z*: 896.2 $[M+NH_4]^+$ (100); HRESIMS *m/z*: 901.2718 $[M+Na]^+$ (calcd for $C_{41}H_{50}O_{21}Na$: 901.2737).

Velutabularin J (**10**): white, amorphous powder; $[\alpha]_{D^{25}}$ −10 (*c* 0.11, CH_3OH); IR (KBr) ν_{max} 3445, 2977, 1741, 1639, 1384, 1246,1031 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 3; negative ESIMS *m/z*: 777.3$[M-H]^-$ (100); positive ESIMS *m/z*: 796.3 $[M+NH_4]^+$ (100); HRESIMS *m/z*: 801.2591 $[M+Na]^+$ (calcd for $C_{37}H_{46}O_{18}Na$: 801.2576).

3.4. Cytotoxicity and anti-inflammatory activity assays

Major compounds (**2**, **4**, **5**, **9**) were evaluated for cytotoxic activity against MCF-7 (human breast cancer), Hela (human cervical cancer), SGC-7901 and BGC-823 (human gastric cancer), HepG2 (human liver cancer), cells by an MTT assay as described in the literature.[10] The cells were obtained from the Cell Bank of the Shanghai Institute of Cell Biology. Taxol was used as a positive control, and the experiments were conducted for three independent replicates.

The anti-inflammatory activities of them were evaluated for inhibitory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in macrophage (RAW264.7) cell line (obtained from the Cell Bank of the Shanghai Institute of Cell Biology). The level of NO was determined using the NO kit (Nanjing Jiancheng Bioengineering Institute) according to the manufacturing's protocol. Dexamethasone was used as positive control substance, and the experiments were conducted for three independent replicates.

Acknowledgements

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Supplementary data

The proposed biosynthetic pathway, ^1H NMR, ^{13}C NMR, and HRESIMS spectra of compounds **1**–**10** can be found in Supplementary data. Supplementary data related to this article can be found online at doi:10.1016/j.tet.2011.02.049.

References and notes

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