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Grayanane diterpenoids from the leaves of *Craiobiodendron yunnanense*

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Abstract—Ten new grayanane diterpenoids, craiobiotoxins I–VIII (**1**–**8**) and craiobiosides A (**9**) and B (**10**), and five known grayanane diterpenoids, grayanotoxin XVIII, lyoniol A, lyoniol B, pieristoxin H, and grayanoside B were isolated from the leaves of *Craiobiodendron yunnanense* (Ericaceae). The structures of **1**–**10** were elucidated by spectroscopic methods, including various 2D NMR and X-ray crystal diffraction experiments. In biological testing of our isolates, craiobiotoxin III (**3**) and lyoniol B showed moderate antifeedant activity.

1. Introduction

Grayanane-type diterpenoids possess a unique structural frame with 5/7/6/5 (trans or cis/cis/cis) ring system, formed probably by rearrangement of the kaurane skeleton. They have been found particularly in some poisonous species of the genera *Kalmia*, *Leucothoe*, *Lyonia*, *Pieris*, and *Rhododendron* of the Ericaceae family.[1] All these diterpenoids contain highly oxygenated functionalities including four more hydroxyl groups. They are responsible for increasing the membrane permeability for sodium ions at normal resting potential via modification of gating properties of sodium channels.[2] Some of them revealed significant biological properties, including potent acute toxicity in mammals[3,4] and antifeedant, growth inhibitory, and insecticidal activities.[5,6] Previously, we investigated these diterpenoids from the species *Rhododendron molle*, *Pieris japonica*, and *Pieris formosa*.[7–14]

In our continuing search for diterpenoids with structural diversity and biological importance from Ericaceae plants, we investigated *Craiobiodendron yunnanense*, a well-known poisonous species in the family. The genus *Craiobiodendron* W. W. Smith is very small group, distributed limitedly in Southeast Asia. Previous studies reported that two grayanane diterpenoids rhodojaponins II and III were isolated from the leaves of *Craiobiodendron henry*,[15] and several flavonoids from the leaves of *C. yunnanense*.[16,17] Species *C. yunnanense* is an evergreen shrub or tree, indigenous to Yunnan province of China. It has been described that eating two pieces of the plant leaves would put people in a coma state for more than 1 day.[18] The dried plant leaves have been used as Chinese folk medicine for relieving pain of arthritis, stomachalgia, paralysis, and as insecticides in southwest region.[19] In the course of our study on the leaves, we have isolated 10 new and five known grayanane-type diterpenoids. This paper describes the isolation and structural elucidation of 10 new grayanane-type diterpenoids craiobiotoxins I–VIII (**1**–**8**), and craiobiosides A (**9**) and B (**10**) on the basis of spectral data and X-ray diffraction analysis. The results of antifeedant bioassay of isolated diterpeoids were also reported.

2. Results and discussion

Craiobiotoxin I (**1**), a colorless oil, exhibited a molecular formula of $C_{20}H_{32}O_5$, as deduced from its HRESIMS and NMR data. The IR spectrum indicated the presence of hydroxyl (3400 cm^{-1}) and double bond (1625 cm^{-1}) functionalities. The ^1H NMR spectrum (Table 1) contained signals for two singlet methyls (δ 1.15, 1.60), two oxygenated methines (δ 3.97, 4.02), one oxygenated methylene (δ 4.04, 4.16) and two exocyclic olefinic protons (δ 5.17, 5.18). Altogether, 20 carbon signals were

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Keywords: Ericaceae; *Craiobiodendron yunnanense*; Grayanane diterpenoids; Craiobiotoxins I–VIII; Craiobiosides A and B; Antifeedant.

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Table 1. ^1H NMR data of compounds **1**–**10** in C_5D_5N[a]

Position	**1**	**2**	**4**	**5**	**6**
1	3.13, dd, 9.6, 9.1	2.83, s	3.01, s	2.82, d, 3.4	3.40, d, 10.7
2α	2,25, ddd, 2.7, 9.1, 14.0	4.22, d, 2.6	4.17, d, 2.0	4.63, dd, 3.4, 4.9	5.10, dd, 3.7, 10.7
2β	2.60, ddd, 6.6, 9.6, 14.0				
3	3.97, dd, 2.7, 6.6	3.27, d, 2.6	3.28, d, 2.0	4.00, d, 4.9	3.93, d, 3.7
6	4.02, dd, 2.2, 9.6	4.04, dd, 3.9, 9.0	4.02, d, 9.2	4.26, d, 9.2	4.14, dd, 4.4, 11.4
7α	1.90, dd, 2.2, 12.2	2.20, dd, 13.3, 9.0		1.88, d, 13.5	2.70, dd, 13.3, 11.4
7β	2.58, dd, 9.6, 12.4	2.69, dd, 3.9, 13.3	6.10, d, 9.2	2.73, dd, 13.5, 9.2	2.31, dd, 4.4, 13.3
9	2.83, br s	1.83, d, 6.6		2.01, br s	2.01, d, 6.5
11α	1.70, m	2.11, m	2.58, m	1.76, m	2.37, m
11β	1.65, m	1.75, m	1.81, m	1.67, m	1.81, m
12α	1.73, m	2.52, m	2.63, m	1.77, m	2.51, m
12β	1.79, m	1.62, m	1.71, m	1.62, m	1.73, m
13	2.52, br s	2.35, br s	2.36, br s	2.21, br s	2.51, br s
14α	1.88, d, 11.1	2.26, d, 11.1	2.55, d, 10.3	1.87, d, 10.9	2.49, d, 10.5
14β	2.32, dd, 4.0, 11.1	2.46, dd, 4.0, 11.1	3.04, dd, 3.1, 10.3	2.30, dd, 4.0, 10.9	2.57, dd, 4.0, 10.5
15α	2.07, d, 14.2	1.99, d, 14.3	2.20, d, 15.1	2.19, d, 14.3	2.06, d, 14.3
15β	2.15, d, 14.2	2.03, d, 14.3	2.78, d, 15.1	2.12, d, 14.3	2.01, d, 14.3
17	4.04, 4.16, each d, 11.0	1.55, s	1.50, s	1.56, s	1.65, s
18	1.15, s	1.32, s	1.33, s	1.36, s	1.35, s
19	1.60, s	1.57, s	1.62, s	1.58, s	1.57, s
20	5.17, 5.18, each, s	1.88, s	2.14, s	5.37, 5.90, each, s	1.88, s
OAc			2.18, s		
Position	**3**	**7**	**8**	**9**	**10**
1	3.00, s	2.84, d, 9.1	3.22, d, 7.7	2.83, d, 8.7	3.12, d, 8.3
2	4.17, d, 2.3	4.86, dd, 4.0, 9.1	5.03, dd, 7.7, 3.0	4.88, dd, 4.3, 8.7	4.96, dd, 4.4, 8.3
3	3.24, d, 2.3	3.93, d, 4.0	4.00, d, 3.0	4.33, d, 4.3	4.33, d, 4.4
6	4.10, d, 10.3	4.04, d, 11.4	4.00, d, 9.8	4.02, d, 10.7	4.02, d, 10.5
7α	2.52, dd, 10.3, 13.3	2.04, dd, 13.3, 11.4	1.98, d, 13.5	2.02, d, 13.5	1.97, d, 13.8
7β	2.69, d, 13.3	3.45, dd, 10.0, 13.5	3.31, dd, 13.5, 9.8	3.46, dd, 13.5, 10.7	3.33, dd, 13.8, 10.5
9	2.00, br s	1.98, d, 6.5	2.32, d, 6.2	2.30, d, 4.7	2.30, d, 4.7
11α	2.11, m	2.77, m	1.80, m	2.32, m	1.82, m
11β	1.75, m	1.81, m	1.70, m	1.83, m	1.68, m
12α	2.82, m	2.51, m	1.92, m	2.50, m	1.90, m
12β	1.58, m	1.73, m	1.68, m	1.74, m	1.68, m
13	2.05, br s	2.25, br s	2.23, br s	2.25, m	2.30, br s
14α	6.16, s	2.12, d, 10.5	2.14, d, 11.0	2.21, d, 11.3	2.18, d, 10.9
14β		2.38, dd, 4.0, 10.5	2.36, dd, 4.4, 11.0	2.43, dd, 3.8, 10.9	2.35, dd, 4.0, 10.9
15α	2.28, d, 14.7	2.06, d, 14.3	2.09, d, 14.0	2.11, d, 14.0	2.08, d, 14.0
15β	2.16, d, 14.7	1.90, d, 14.3	1.90, d, 14.0	1.83, d, 14.0	1.86, d, 14.0
17	1.46, s	1.65, s	1.56, s	1.61, s	1.56, s
18	1.36, s	1.21, s	1.33, s	1.32, s	1.35, s
19	1.57, s	1.76, s	1.82, s	1.96, s	1.93, s
20	1.88, s	1.51, s	5.64, 5.40, each, s	1.75, s	5.51, 5.39, each, s
1′				5.17, d, 7.9	5.19, d, 7.8
2′	2.82, m			4.23, m	4.22, m
3′	4.23, m			4.12, m	4.12, m
4′	1.28, d, 6.9			4.44, m	4.44, m
5′	1.33, d, 6.0			4.10, m	4.15, m
6′				4.64, dd, 1.6, 11.5 4.42, dd, 5.5, 11.5	4.65, dd, 2.2, 11.8 4.42, dd, 5.7, 11.8

[a] In ppm relative to internal TMS run at 400 MHz. Assignments are based on BB, DEPT, HSQC, ^1H–^1H COSY, HMBC, and NOESY experiments.

observed in the ^{13}C NMR (DEPT) spectrum (Table 2), including two methyl, eight methylenes (one olefinic at δ 112.5, one oxygenated at δ 66.7), five methines (two oxygenated at δ 71.0, 81.5), and five quaternary carbons (one olefinic at δ 153.0 and two oxygenated at δ 83.4, 83.7). The ^1H–^1H COSY spectrum revealed the presence of the following fragments: CH–CH_2–CH(OH), CH(OH)–CH_2, and CH–CH_2–CH_2–CH–CH_2. These structural features suggested that **1** was a grayanane-type diterpenoid with five sites of oxygenation. Further studies on the ^1H and ^{13}C NMR spectra of **1** revealed that these data closely resembled those of grayanotoxin XVIII,[20] a known diterpenoid isolated from the same species by us. The only difference was that **1** has an oxygenated methylene (H_2-17, δ 4.04, 4.16, each 1H, d, $J = 11.0$ Hz; C-17, δ 66.7 t) but lacked a β-methyl like C-17 of grayanotoxin XVIII, suggesting **1** is a C-17 oxygenated derivative of grayanotoxin XVIII. This proposal was supported by HMBC correlations between C-17/H-15; C-15/H-17, C-13/H-17, and also substantiated by the downfield shift of C-16 (δ 83.4 in **1** and δ 79.3 in grayanotoxin XVIII), upfield shift of C-15 (δ 58.5 in **1**

Table 2. ^{13}C NMR data of compounds **1**–**10** in C_5D_5N[a]

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	44.4 d	54.4 d	54.3 d	55.6 d	47.0 d	52.0 d	70.9 d	68.0 d	70.6 d	67.9 d
2	39.7 t	60.3 d	60.3 d	59.6 d	77.8 d	75.1 d	74.8 d	74.2 d	73.8 d	73.1 d
3	81.5 d	64.3 d	64.3 d	64.5 d	81.7 d	84.1 d	83.4 d	84.6 d	91.2 d	92.6 d
4	50.8 s	48.0 s	48.0 s	48.6 s	49.3 s	47.9 s	48.0 s	48.7 s	48.8 s	49.4 s
5	83.7 s	80.1 s	79.9 s	80.5 s	84.5 s	84.3 s	85.9 s	86.6 s	85.0 s	85.7 s
6	71.0 d	74.4 d	73.3 d	79.3 d	70.7 d	75.2 d	70.6 d	71.2 d	70.8 d	71.5 d
7	46.9 t	50.2 t	44.2 t	78.2 d	47.0 t	50.5 t	49.8 t	48.8 t	49.9 t	48.8 t
8	44.3 s	46.4 s	50.7 s	57.4 s	50.7 s	50.7 s	46.2 s	45.9 s	46.2 s	45.9 s
9	52.7 d	53.4 d	55.8 d	80.3 s	53.0 d	55.2 d	59.0 d	55.7 d	58.2 d	55.7 d
10	153.0 s	77.7 s	77.4 s	81.1 s	149.0 s	77.4 s	78.1 s	152.9 s	78.1 s	152.4 s
11	26.1 t	23.2 t	22.4 t	32.4 t	24.5 t	22.4 t	19.7 t	26.9 t	19.4 t	26.9 t
12	23.7 t	26.5 t	27.3 t	26.9 t	25.6 t	26.9 t	27.3 t	26.8 t	27.4 t	26.8 t
13	44.8 d	51.1 d	55.4 d	50.0 d	48.3 d	55.4 d	49.6 d	49.9 d	49.7 d	50.0 d
14	36.2 t	35.6 t	81.9 d	30.0 t	36.4 t	36.3 t	37.7 t	35.8 t	37.7 t	35.9 t
15	58.5 t	61.0 t	61.1 t	50.5 t	62.3 t	61.5 t	60.5 t	57.4 t	60.5 t	57.4 t
16	83.4 s	78.2 s	78.6 s	77.5 s	79.7 s	78.6 s	78.5 s	78.7 s	78.1 s	80.0 s
17	66.7 t	24.6 q	24.1 q	24.4 q	25.7 q	24.1 q	24.9 q	24.9 q	24.8 q	25.0 q
18	24.5 q	21.4 q	21.4 q	21.8 q	28.1 q	23.3 q	21.9 q	22.2 q	22.6 q	23.2 q
19	19.5 q	20.7 q	20.6 q	21.0 q	20.6 q	20.2 q	21.6 q	21.2 q	21.8 q	21.5 q
20	112.5 t	31.0 q	30.8 q	27.7 q	115.2 t	31.5 q	25.4 q	110.1 t	25.8 q	110.2 t
1′			175.5 s	171.9 s					104.6 d	104.7 d
2′			49.1 d	22.0 q					75.2 d	75.4 d
3′			69.9 d						78.7 d	78.8 d
4′			24.8 q						71.7 d	71.8 d
5′			14.3 q						79.0 d	79.0 d
6′									62.7 d	62.8 d

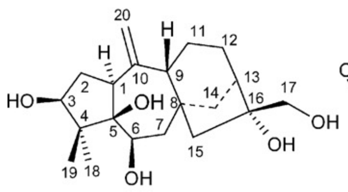
[a] In ppm relative to internal TMS run at 125 MHz. Assignments are based on BB, DEPT, HSQC, ^1H–^1H COSY, and HMBC experiments.

and δ 62.1 in grayanotoxin XVIII) and C-13 (δ 44.8 in **1** and δ 47.6 in grayanotoxin XVIII), and no NOESY correlation between H_2-17 and H-13. Further analysis on HMBC, HMQC, and NOESY data enabled full assignments of the ^1H and ^{13}C NMR data of **1**. Therefore, compound **1** was deduced as 3β,5β,6β,16α,17-pentahydroxy-grayan-10(20)-ene, named craiobiotoxin I. This is the first report of C-17 oxygenated grayanane diterpenoid.

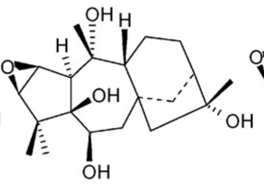
Craiobiotoxin II (**2**), a colorless crystal, gave a molecular formula of $C_{20}H_{32}O_5$, as found from its HRESIMS and NMR data. The IR spectrum showed the presence of hydroxyl (3445 cm^{-1}) group. The ^1H NMR spectrum (Table 1) contained signals for four singlet methyls (δ 1.32, 1.55, 1.57, 1.88) and three oxygenated methines (δ 3.27, 4.04, 4.22). The ^{13}C NMR (DEPT) spectrum (Table 2) revealed 20 carbon signals for four methyls, five methylenes, six methines (three oxygenated at δ 60.3, 64.3, 74.4), and five quaternary carbons (three oxygenated at δ 77.7, 78.2, 80.1). The aforementioned ^1H and ^{13}C NMR data are very similar with those of a known diterpenoid rhodojaponin III.[5] The only difference was that **2** has an upfield methylene (H_2-14, δ 2.26, 2.46; C-14, δ 35.6 t) instead of an oxygenated methine in rhodojaponin III (H-14, δ 4.90 s; C-14, δ 78.9 d), suggesting that **2** is 14-dehydroxy-rhodojaponin III. This proposal was confirmed by 2D NMR experiments and X-ray diffraction analysis (Fig. 1). Thus, **2** was determined as 2,3-epoxy-5β,6β,10α,16α-tetrahydroxy-grayanane, named craiobiotoxin II.

Craiobiotoxin III (**3**), a colorless crystal, has a molecular formula of $C_{25}H_{40}O_8$, as deduced from its HRESIMS and NMR data. The IR spectrum showed the presence of hydroxyl (3450 cm^{-1}) and ester carbonyl (1725 cm^{-1}) functionalities. The ^1H NMR spectrum (Table 1) contained signals for four singlet methyls (δ 1.36, 1.46, 1.57, 1.88), two doublet methyls (δ 1.28, 1.33), and five oxygenated methines (δ 3.24, 4.10, 4.17, 4.23, 6.16). The ^{13}C NMR (DEPT) spectrum (Table 2) revealed 25 carbon signals, including six methyls, four methylenes, nine methines (five oxygenated at δ 60.3, 64.3, 69.9, 73.3, 81.9), and six quaternary carbons (one ester carbonyl at δ 175.5, and three oxygenated at δ 77.4, 78.6, 79.9). Further studies on the ^1H and ^{13}C NMR data suggested that **3** was a derivative of rhodojaponin III, with an ester side-chain composed by a $C_5H_9O_2$ fragment, which was deduced as $-CO-CH(CH_3)-CH(OH)-CH_3$ by ^{13}C NMR and ^1H–^1H COSY spectra. The downfield shift of H-14 (δ 6.16, 1H, s) and C-14 (δ 81.9) and the HMBC correlations between ester carbon with H-14 and H-5′ indicated that C_{14}–OH was esterified. The structure and its relative stereochemistry of **3** were confirmed by X-ray diffraction analysis (Fig. 2). Thus, **3** was determined as 2,3-epoxy-5β,6β,10α,16α-tetrahydroxyl-14-*O*-(2-hydroxyl-3-methyl-butyryl)-grayanane, named craiobiotoxin III.

Craiobiotoxin IV (**4**), a colorless crystal, gave a molecular formula of $C_{22}H_{34}O_8$, as deduced from its HRESIMS and NMR data. The IR spectrum showed the presence of hydroxyl (3450 cm^{-1}), and ester carbonyl (1720 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) contained signals for four singlet methyls (δ 1.33, 1.50, 1.62, 2.14), one acetyl methyl (δ 2.18), and four oxygenated methines (δ 3.28, 4.02, 4.17, 6.10). The ^{13}C NMR (DEPT) spectrum (Table 2) revealed 22 carbon signals



1



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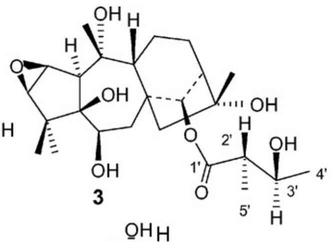

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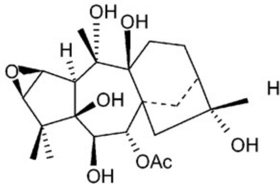

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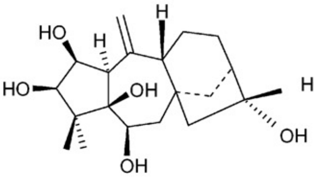

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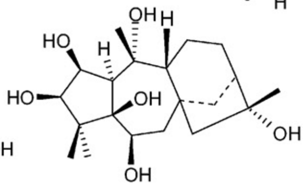

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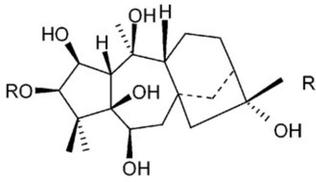
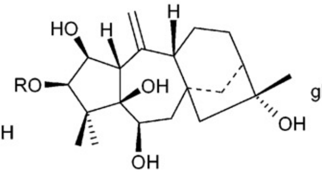
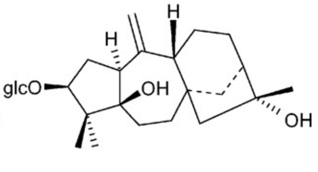


7 R=H **9** R=glc



8 R=H **10** R=glc



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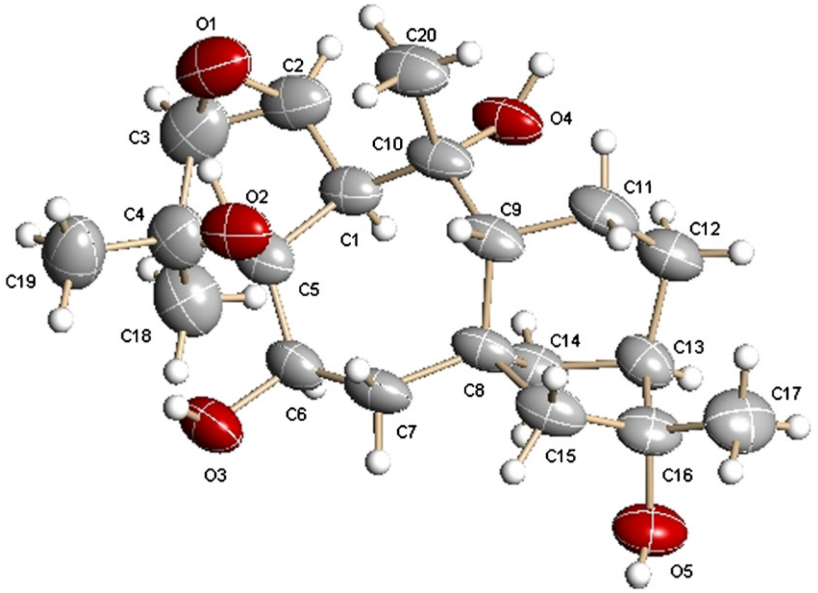

Figure 1. A perspective view of **2** by X-ray diffraction.

including five methyls, four methylenes, six methines (four oxygenated at *δ* 59.6, 64.5, 78.2, 79.3), and seven quaternary carbons (one ester carbonyl at *δ* 171.9, and four oxygenated at *δ* 77.5, 80.3, 80.5, 81.1). The ^1H–^1H COSY spectrum indicated the presence of the following fragments: CH–CH(O)–CH(O), CH(OH)–CH(OAc), and CH_2–CH_2–CH–CH_2. These structural features suggested that **4** was a grayanane-type diterpenoid with eight sites of oxygenation. Further study revealed that the ^1H and ^{13}C NMR data of **4** were very similar to those of lyoniol A,[21] a known diterpenoid obtained from same species by us. The evident difference between **4** and lyoniol A was that **4** had an oxygenated quaternary carbon (C-9, *δ* 81.1 s) instead of a methine in lyoniol A, suggesting that **4** was 9-hydroxyl-lyoniol A. This proposal was supported by HMBC correlations between C-9/H-12, H_2-15, H_3-20, and downfield shift of adjacent C-8, 10, 11; upfield shift of C-14, 15, and further comparison of the NMR data with those of pieristoxin G (9,14-dihydroxyl-lyoniol A).[22] Therefore, **4** was determined as 2,3-epoxy-5*β*,6*β*,9,10α,16α-pentahydroxy-7α-acetoxy-grayanane, named craiobiotoxin IV.

Craiobiotoxin V (**5**), a colorless crystal, exhibited a molecular formula of $C_{20}H_{32}O_5$, as deduced from its HRESIMS and NMR data. The IR showed characteristic absorptions for hydroxyl (3450 cm^{-1}) and double bond (1640 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) contained signals for three singlet methyls

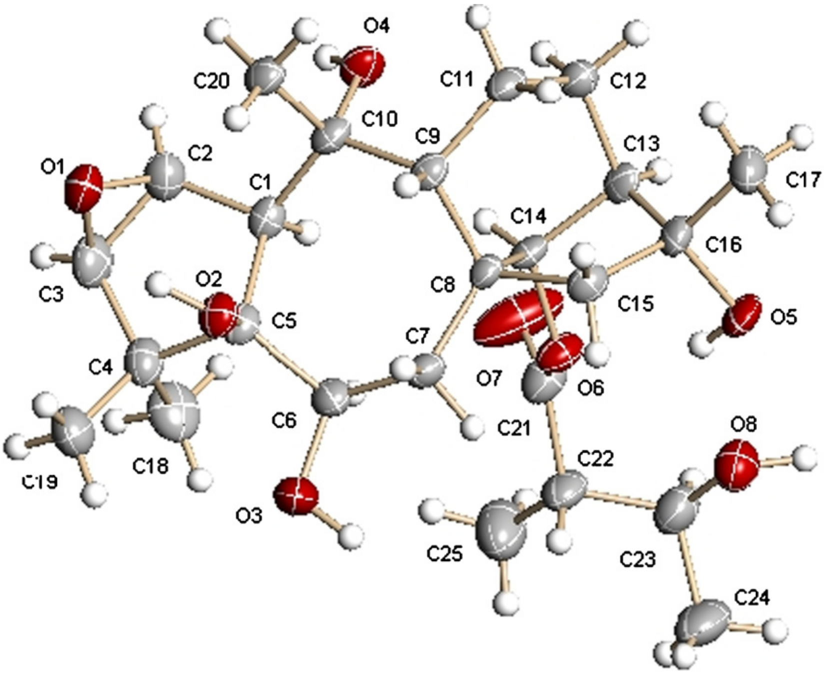

Figure 2. A perspective view of **3** by X-ray diffraction.

(δ 1.36, 1.56, 1.58), three oxygenated methines (δ 4.00, 4.26, 4.63), and two singlet olefinic protons (δ 5.37, 5.90). The ^{13}C NMR (DEPT) spectrum (Table 2) revealed 20 carbon signals for three methyls, six methylenes (one olefinic at δ 115.5), six methines (three oxygenated at δ 70.7, 77.8, 81.7), and five quaternary carbons (one olefinic at δ 149.0 and two oxygenated at δ 79.7, 84.5). The ^{1}H–^{1}H COSY spectrum revealed the presence of the following fragments: CH–CH(OH)–CH(OH), CH(OH)–CH_2, and CH–CH_2–CH_2–CH–CH_2. These structural features suggested that **5** was a grayanane-type diterpenoid with five sites of oxygenation, very similar with those of grayanotoxin XVIII. The only difference is that **2** has a CH–CH(OH)–CH(OH) fragment instead of the corresponding CH–CH_2–CH(OH) in grayanotoxin XVIII, suggesting that **5** was 2-hydroxy-grayanotoxin XVIII. This proposal was supported by various 2D NMR experiments and its relative stereochemistry was decided by X-ray diffraction analysis (Fig. 3). Thus, **5** was determined as 2β,3β,5β,6β,16α-pentahydroxygrayan-10(20)-ene, named craiobiotoxin V.

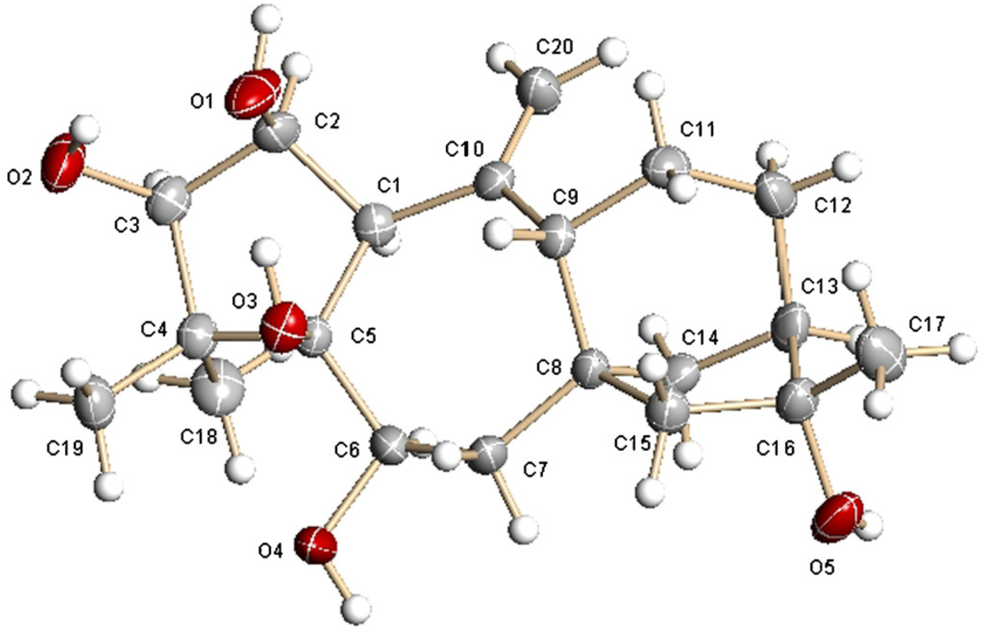

Figure 3. A perspective view of **5** by X-ray diffraction.

Craiobiotoxin VI (**6**), a colorless crystal, was assigned a molecular formula of $C_{20}H_{34}O_6$, as deduced from its HRESIMS and NMR data. The IR spectrum showed the presence of hydroxyl (3443 cm^{-1}) group. The NMR data of **6** (Tables 1 and 2) were very similar to those of **5**. The only difference was that **6** lacked the exocyclic double bond, but appeared the signals for a methyl and an oxygenated quaternary carbon, suggesting that **6** is a hydrated derivative of **5**. This was supported by various 2D NMR data and confirmed by X-ray diffraction analysis (Fig. 4). Thus, **6** was determined as 2β,3β,5β,6β,10α,16α-hexahydroxy-grayanane, named craiobiotoxin VI.

Craiobiotoxin VII (**7**), a colorless crystal, was assigned a molecular formula of $C_{20}H_{34}O_6$, as deduced from its HRESIMS and NMR data. The IR spectrum showed the presence of hydroxyl (3435 cm^{-1}) group. The ^1H and ^{13}C NMR data (Tables 1 and 2) of **7** were very similar to those of **6**. The structural fragments deduced from ^1H–^1H COSY spectrum were same with those of **6**. The obvious difference came from the NMR data of C-1 (δ 70.9 in **7** and δ 52.0 in **6**) and C-20 (δ 25.4 in **7** and δ 31.5 in **6**), suggesting that **7** was an isomer of **6**, with *cis*-junction of A/B rings and α-methyl in C-10. This was supported by NOESY correlation between H-1/H-9 in **7**, comparing to the NOESY correlation between H-1/H-14α in **6**, and X-ray diffraction analysis (Fig. 5). Thus, **7** was determined as 1-*epi*-2β,3β,5β,6β,10β,16α-hexahydroxy-grayanane, named craiobiotoxin VII.

Craiobiotoxin VIII (**8**), a colorless oil, was assigned a molecular formula of $C_{20}H_{32}O_5$, as deduced from its HRESIMS and NMR data. The IR spectrum showed the presence of hydroxyl (3455 cm^{-1}) and double bond

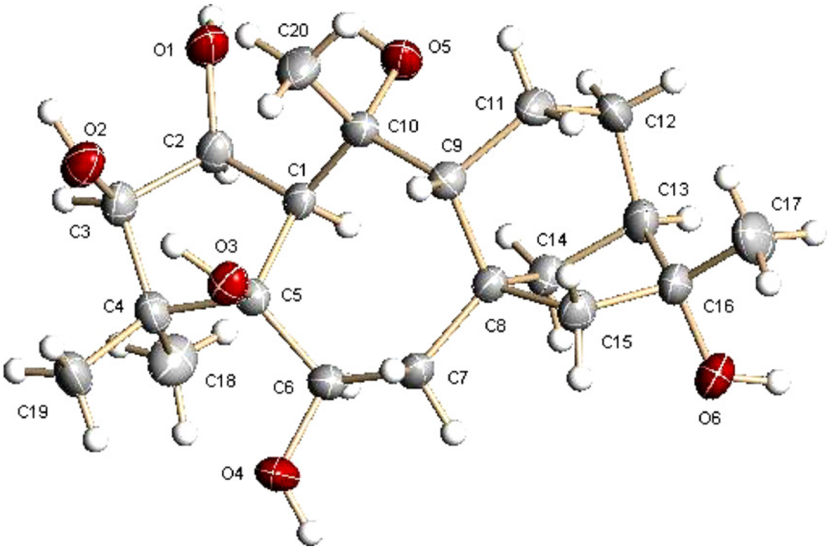

Figure 4. A perspective view of **6** by X-ray diffraction.

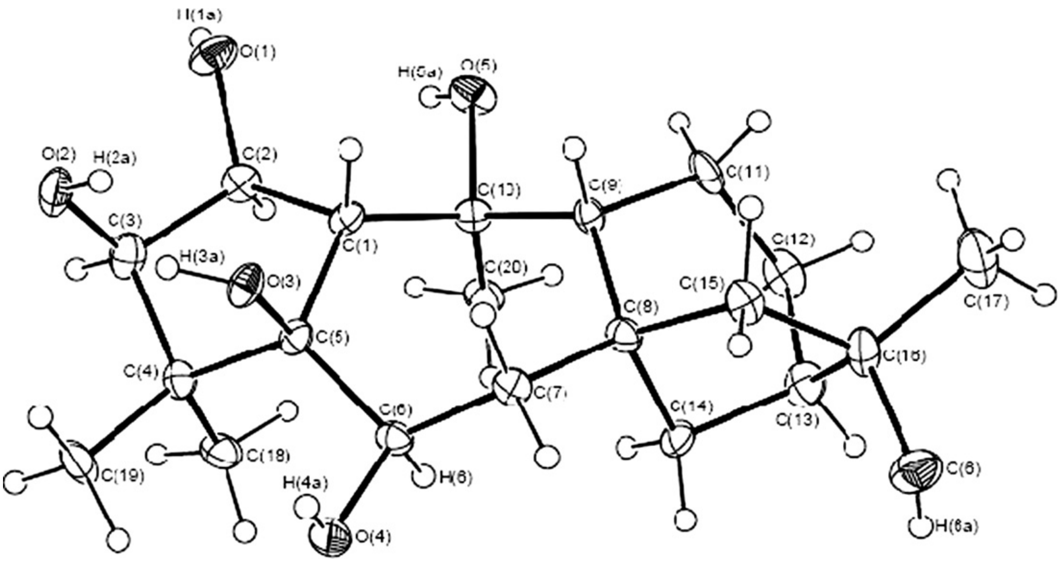

Figure 5. A perspective view of **7** by X-ray diffraction.

(1625 cm^{-1}) functionalities. It was noticed that **8** and **5** had the same molecular formula, same functional groups, same carbon numbers, and multiplicities, same structural fragments, and very similar NMR data (Tables 1 and 2) by analysis of MS, ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, and HMBC data. The only obvious difference between **8** and **5** came from the NMR data of C-1 (δ 68.0 in **8** and δ 47.0 in **5**), suggesting **8** was a C-1 epimer of **5**, having *cis*-junction of A/B rings. This deduction was supported by NOESY correlation between H-1 and H-9 in **8**, comparing to those between H-1 and H-14α in **5**, and further detailed comparison on NMR data of **8** with those of **5**, **6**, and **7**. Thus, **8** was established as 1-*epi*-2β,3β,5β,6β,16α-pentahydroxygrayanan-10(20)-ene, named craiobiotoxin VIII.

Craiobioside A (**9**), a major component of the plant, had a molecular formula $C_{26}H_{44}O_{11}$, as determined by HRESIMS and NMR data. The IR spectrum showed the presence of hydroxyl (3435 cm^{-1}) group. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **9** were very similar to those of **7**, besides **9** had additional signals for seven protons (δ 5.17, 1H, H-1′, 4.12–4.64, 6H, H-2′–H-6′) and six carbons (δ 62.7, 71.7, 75.2, 78.7, 79.0, 104.6), corresponding to a β-glucosyl moiety in pyranose form,[14] as deduced by ^{1}H–^{1}H COSY, HMQC and HMBC experiments and a large coupling constant of anomeric proton H-1′ (δ 5.17, d, $J = 7.9$ Hz). Furthermore, an enzymatic hydrolysis of **9** by cellulase afforded D-glucose detected by co-TLC and an aglycone identified as **7** by spectral analysis. The downfield shift of H-3 (from δ 3.93 in **7** to δ 4.33 in **9**) and C-3 (from δ 83.4 in **7** to δ 91.2 in **9**), and the HMBC correlations of C-3 with H-1′ and C-1′ with H-3, indicated that C_3-OH was glucosylated. Thus, **9** was determined as craiobiotoxin VII 3-*O*-β-D-glucopyranoside, named craiobioside A.

Craiobioside B (**10**), a major component of the plant, had a molecular formula $C_{26}H_{42}O_{10}$, as determined by HRESIMS and NMR data. The IR spectrum showed the presence of hydroxyl (3500 cm^{-1}) group. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) were very similar to those of **8**, besides **10** had additional signals for seven protons (δ 5.19, 1H, H-1′, 4.12–4.65, 6H, H-2′–H-6′) and six carbons (δ 62.8, 71.8, 75.4, 78.8, 79.0, 104.7), corresponding to a β-glucosyl moiety in pyranose form,[14] as deduced by ^{1}H–^{1}H COSY, HMQC, and HMBC experiments and a large coupling constant of anomeric proton H-1′ (δ 5.19, d, $J = 7.8$ Hz). The enzymatic hydrolysis of **10** by cellulase afforded D-glucose detected by co-TLC and an aglycone identified as **8** by spectral analysis. The downfield shift of H-3 (from δ 4.00 in **8** to δ 4.33 in **10**) and C-3 (from δ 84.6 in **8** to δ 92.6 in **10**), and the HMBC correlations of C-3 with H-1′ and C-1′ with H-3, indicated that C_3-OH was glucosylated. Thus, **10** was determined as craiobiotoxin VII 3-*O*-β-D-glucopyranoside, named craiobioside B.

All other five known compounds from the leaves were identified as grayanane-type diterpenoids as grayanotoxin XVIII,[20] lyoniol A,[21] lyoniol B,[21] pieristoxin H,[22] and grayanoside B[20] by comparison of their spectral data with literature values. The grayanane diterpenoids are highly oxygenated in the form of hydroxyl, ketone, ester, and ether functionalities. A literature investigation indicated that the oxygenation could occur at any position of C-2, 3, 5, 6, 7, 9, 10, 14, and 16. The hydroxyl groups, commonly occurring functionality in grayanane diterpenoids, were found to have orientation in 3β,5β,6β,7α,9β,14β,16α and either 2α and 2β, or 10α and 10β forms. The A/B ring junction could be in *cis* or *trans* form while the B/C/D ring system had a fixable junction in *cis/cis/cis* form. Up to now more than 70 grayanane diterpenoids have been reported. However, the determination of A/B junction in a few reported diterpenoids is still ambiguous due to lack of reliable approach to differentiate either of *trans* or *cis* junction. Though an observation on NOESY correlation between H-1/H-14α is commonly used to determine an A/B *trans* junction, a confused judgment has often been made due to proton signals overlapping. In our present study, we obtained **5** and **8**, a pair of C-1 epimers, which enable us to find an obvious difference of the NMR data arising from the A/B ring junction. In combination with literature investigation, it is noticed that the chemical shift of C-1 in H-1β grayanane diterpenoids moved downfield for 15–20 ppm in comparison to those of H-1α, for example, δ 68.0 in **8** (H-1β) and δ 47.0 in **5** (1-*epi* **8**), δ 59.0 in grayanoside C[23] (H-1β) and δ 43.1 in grayanoside B (1-*epi* grayanoside C), δ 58.0 in grayathol A[23] (H-1β) and δ 43.5 in grayanotoxin XIX (1-*epi* grayathol A)[1,24]. The above observation can be used for determining an A/B ring junction. Previously, pierisformoside B[12] was reported to have a *cis* form for A/B ring system. However, its chemical shift value of C-1 at δ 50.6 was different from that of grayathol A[23] (H-1β) at δ 58.0, but close to that of 6-dehydroxyl-grayanotoxin II[25] at δ 47.7, suggesting that the A/B ring junction of pierisformoside B should be revised from *cis* (H-1β) to *trans* (H-1α) form as **11** by analysis of ^{13}C NMR data.

All the isolated compounds are sent out for performing the antifeedant bioassay. The results show that craiobiotoxin III (**3**) and lyoniol B are moderately active. Compared to the LC_{50} value of azidirachtin (2 ppm), the LC_{50} of **3** and lyoniol B is 37 and 10 ppm, respectively, while those of other compounds are more than 50 ppm.

3. Materials and methods

3.1. General

Optical rotations were measured on a Perkin-Elmer MC-241 polarimeter. IR spectra were measured on a Nicolt-Magna 750 spectrophotometer. Mass spectra were recorded using a MAT-241 mass spectrometer. NMR spectra were recorded on Bruker AM-400 or 500 NMR spectrometers.

3.2. Plant material

The leaves of *Craiobiodendron yunnanensis* were collected in Dali, Yunnan Province, People's Republic of China, and identified by Prof. Li Yin of the Department of

Botany, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen has been deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The air-dried and powdered leaves of *C. yunnanensis* (10 kg) were extracted with 95% EtOH (3 × 40 L) three times at room temperature. The combined residue, after removal of solvent, was extracted by $CHCl_3$, EtOAc, and *n*-BuOH, successively. The *n*-BuOH extracts (800 g) were applied to a resin 101 column (5 kg) and eluted with H_2O, 50% EtOH, and 95% EtOH, sequentially. The 95% EtOH fractions (100 g) were subjected to passage over a silica gel column (200–300 mesh, 800 g) and eluted with $CHCl_3$/MeOH (20:1) [Part B], $CHCl_3$/MeOH (10:1) [Part B], $CHCl_3$/MeOH (5:1) [Part C], and $CHCl_3$/MeOH/H_2O (4:1:0.1) [Part D]. Repeated column chromatography on silica gel H, Sephadex LH-20, and Lichroprep RP-18 afforded craiobiotoxins I (**1**, 15 mg), II (**2**, 28 mg), and grayanotoxin XVIII (50 mg) from Part B: craiobiotoxins III (**3**, 15 mg), IV (**4**, 25 mg), V (**5**, 15 mg), VI (**6**, 10 mg), VII (**7**, 12 mg), VIII (**8**, 17 mg), peristoxin H (4 mg), and lyoniols A (9 mg), B (200 mg) from Part C; and craiobiosides A (**9**, 1.5 g), B (**10**, 2.0 g), and grayanoside B (2.2 g) from Part D.

3.4. Insect antifeedant bioassay

Chronic feeding bioassays were carried out with freshly hatched larvae ($n = 20$) of the polyphagous pest insect *Spodoptera littoralis* (Noctuidae, Lepidoptera). The larvae were from a laboratory colony reared on artificial diet under controlled conditions at 26 °C as described previously.[5] The test insects were kept on artificial diet spiked with four different concentrations of pure compounds (1–50 ppm) which were applied with MeOH. After five days (moist chamber, darkness, 26 °C) the survival rate and the larval growth of the surviving larvae were monitored in comparison to controls that had been exposed to diet treated with solvent only. LC_{50} and EC_{50} values were calculated by probit-log analysis.

3.5. Enzymatic hydrolysis of 9 and 10

A mixture of **9** (30 mg) and commercial cellulase (200 mg) in water solution (15 ml) was reacted for 48 h. The D-glucose in the mixture was detected by TLC using *n*-BuOH/EtOAc/*iso*-PrOH/HOAc/H_2O (7:20:12:3:6) solvent system to develop with standard sugars. The reaction product was successively extracted with two portions (50 mL) of *n*-BuOH, and the extract chromatographed on silica gel, to afford compound **7** (20 mg). The same method using a mixture of **10** (30 mg) and cellulase (200 mg) afford compound **8** (21 mg).

3.6. Compounds 1–10

3.6.1. Craiobiotoxin I (1). IR (KBr) ν_{max} (cm^{-1}): 3400 (OH), 2950, 1625, 1380, 1010. ESI-MS: *m/z* 375 $[M+Na]^+$, 351 $[M-H]^+$, HRESI-MS: 375.2140 $[M+Na]^+$ ($C_{20}H_{32}O_5Na$, Calcd 375.2147); ^1H and ^{13}C NMR data see Tables 1 and 2.

3.6.2. Craiobiotoxin II (2). mp: 115–117 °C; IR (KBr) ν_{max} (cm^{-1}): 3445 (OH), 2960, 1370, 1030, 1010. ESI-MS: *m/z* 375 $[M+Na]^+$, 351 $[M-H]^+$, HRESI-MS: 375.2145 $[M+Na]^+$ ($C_{20}H_{32}O_5Na$, Calcd 375.2147); ^1H and ^{13}C NMR data see Tables 1 and 2.

3.6.3. Crystallographic data of compound 2. The structure of **2** was unambiguously determined by single-crystal X-ray diffraction technique. A suitable crystal of compound **2** obtained by recrystallization from $CHCl_3$ and MeOH (10:1). A colorless crystal with dimensions $0.418 \times 0.201 \times 0.182\ mm^3$ was selected for the crystallographic measurements. $C_{20}H_{32}O_5$: M_r 352.46, orthorhombic, $a = 11.7135(16)$, $b = 12.5919(17)$, $c = 12.6838(17)$ Å , $\beta = 90°$, $V = 1870.8(4)$ Å^3, space group = P_2, $Z = 4$, $D_{calc} = 1.251\ mg/m^3$, $F(0\ 0\ 0) = 768$, Mo-K$\lambda = 0.71073$ Å. Unit cell dimensions were determined by least-squares fit of 4309 reflections measured at 293 (2) K using a Mo-Kα radiation on a Nonius kappaCCD diffractiometer. The intensity data within θ range of 2.28–28.31° were collected at 173.2 (2) K and the completeness to θ (28.31°) is 95.9%. A total of 11,310 reflections were collected, of which 4309 reflections were judged observed on the basis of $I > 2\sigma(I)$. The structure was solved by the direct methods and expanded using Fourier transformation techniques and refined by a full-matrix least-squares calculation on F^2 with the aid of program SHELXL97.[26] The final R and R_w factors were measured as 0.0596 and 0.1177, respectively. The figures were plotted with the aid of ORTEPII program.[27] Crystallographic data for compound **2** was deposited in Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

3.6.4. Craiobiotoxin III (3). mp: 135–137 °C; IR (KBr) ν_{max} (cm^{-1}): 3450 (OH), 2968, 1725, 1375, 1230, 1050. ESI-MS: *m/z* 491 $[M+Na]^+$, 467 $[M-H]^+$, HRESI-MS: 491.2630 $[M+Na]^+$ (Calcd 491.2621); ^1H and ^{13}C NMR data see Tables 1 and 2.

3.6.5. Crystallographic data of compound 3. The structure of **3** was unambiguously determined by single-crystal X-ray diffraction technique. A suitable crystal of compound **3** was obtained by recrystallization from $CHCl_3$ and MeOH (10:1). A colorless crystal with dimensions $0.423 \times 0.158 \times 0.121\ mm^3$ was selected for the crystallographic measurements. $C_{25}H_{40}O_8$: M_r 468.57, orthorhombic, $a = 6.4031(7)$, $b = 17.298(2)$, $c = 21.487(3)$ Å, $\beta = 90°$, $V = 2379.9(5)$ Å^3, space group = P_2, $Z = 4$, $D_{calc} = 1.308\ mg/m^3$, $F(0\ \ 0\ \ 0) = 1016$, Mo-K$\lambda = 0.71073$ Å. Unit cell dimensions were determined by least-squares fit of 5560 reflections measured at 293 (2) K using a Mo-Kα radiation on a Nonius kappaCCD diffractiometer. The intensity data within θ range of 1.51–28.31° were collected at 173.2 (2) K and the completeness to θ (28.31°) is 96.5%. A total of 14,605 reflections were collected, of which 5560 reflections were judged observed on the basis of $I > 2\sigma(I)$. The structure was solved by the direct methods and expanded using Fourier transformation techniques and refined by a full-matrix least-squares calculation on F^2 with the aid of program SHELXL97.[26] The final R and R_w factors were measured as 0.0643 and 0.1062, respectively. The figures were plotted with the aid of ORTEPII program.[27] Crystallographic data for compound **3** was deposited in Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

3.6.6. Craiobiotoxin IV (4). mp: 146–147 °C, IR (KBr) ν_{max} (cm^{-1}): 3450 (OH), 2968, 1720, 1370, 1230, 1055. ESI-MS: *m*/*z* 449 [M+Na]$^+$, 425 [M−H]$^+$, HRESI-MS: 449.2141 [M+Na]$^+$ ($C_{22}H_{34}O_8Na$, Calcd 449.2151); ^{1}H and ^{13}C NMR data see Tables 1 and 2.

3.6.7. Craiobiotoxin V (5). mp: 137–139 °C; IR (KBr) ν_{max} (cm^{-1}): 3450 (OH), 2970, 1640, 1375, 1233, 1050. ESI-MS: *m*/*z* 375 [M+Na]$^+$, 351 [M−H]$^+$, HRESI-MS: 375.2146 [M+Na]$^+$ ($C_{20}H_{32}O_5Na$, Calcd 375.2147); ^{1}H and ^{13}C NMR data see Tables 1 and 2.

3.6.8. Crystallographic data of compound 5. The structure of **5** was unambiguously determined by single-crystal X-ray diffraction technique. A suitable crystal of compound **5** obtained by recrystallization from $CHCl_3$ and MeOH (10:1). A colorless crystal with dimensions 0.512 × 0.459 × 0.365 mm^3 was selected for the crystallographic measurements. $C_{20}H_{32}O_5$: M_r 352.46, orthorhombic, $a = 6.9899(5)$, $b = 11.4012(2)$, $c = 22.3745(3)$ Å, $\beta = 90°$, $V = 1783.1(2)$ Å^3, space group = $P2(1)2(1)2(1)$, $Z = 4$, $D_{calc} = 1.313$ mg/m^3, $F(0\ 0\ 0) = 768$, Mo-Kλ = 0.71073 Å. Unit cell dimensions were determined by least squares fit of 4153 reflections measured at 293 (2) K using a Mo-Kα radiations on a Nonius kappaCCD diffractometer. The intensity data within θ range of 1.82–28.28° were collected at 173.2 (2) K and the completeness to θ (28.28°) is 96.1%. A total of 10,874 reflections were collected, of which 4153 reflections were judged observed on the basis of $I > 2\sigma(I)$. The structure was solved by the direct methods and expanded using Fourier transformation techniques and refined by a full-matrix least-squares calculation on F^2 with the aid of program SHELXL97.[26] The final R and R_w factors were measured as 0.0429 and 0.0857, respectively. The figures were plotted with the aid of ORTEPII program.[27] Crystallographic data for compound **5** was deposited in Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

3.6.9. Craiobiotoxin VI (6). mp: 162–163 °C; IR (KBr) ν_{max} (cm^{-1}): 3443 (OH), 2966, 1380, 1253, 1045. ESI-MS: *m*/*z* 393 [M+Na]$^+$, 369 [M−H]$^+$, HRESI-MS: 393.2255 [M+Na]$^+$ ($C_{20}H_{34}O_6Na$, Calcd 393.2253); ^{1}H and ^{13}C NMR data see Tables 1 and 2.

3.6.10. Crystallographic data of compound 6. The structure of **6** was unambiguously determined by single-crystal X-ray diffraction technique. A suitable crystal of compound **6** obtained by recrystallization from $CHCl_3$ and MeOH (10:1). A colorless crystal with dimensions 0.505 × 0.457 × 0.356 mm^3 was selected for the crystallographic measurements. $C_{20}H_{34}O_6$: M_r 370.447, orthorhombic, $a = 20.778(3)$, $b = 6.1706(8)$, $c = 16.556(2)$ Å, $\beta = 117.104°$, $V = 1889.7(4)$ Å^3, space group = C_2, $Z = 4$, $D_{calc} = 1.302$ mg/m^3, $F(0\ 0\ 0) = 808$, Mo-Kλ = 0.71073 Å. Unit cell dimensions were determined by least-squares fit of 3895 reflections measured at 293(2) K using a Mo-Kα radiations on a Nonius kappaCCD diffractometer. The intensity data within θ range of 2.00–28.22° were collected at 173.2(2) K and the completeness to θ (28.22°) is 93.8%. A total of 5713 reflections were collected, of which 3895 reflections were judged observed on the basis of $I > 2\sigma(I)$. The structure was solved by the direct methods and expanded using Fourier transformation techniques and refined by a full-matrix least-squares calculation on F^2 with the aid of program SHELXL97.[26] The final R and R_w factors were measured as 0.0505 and 0.1188, respectively. The figure was plotted with the aid of ORTEPII program.[27] Crystallographic data for compound **6** was deposited in Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

3.6.11. Craiobiotoxin VII (7). mp: 165–167 °C; IR (KBr) ν_{max} (cm^{-1}): 3435 (OH), 2966, 1380, 1255, 1050. ESI-MS: *m*/*z* 393 [M+Na]$^+$, 369 [M−H]$^+$, HRESI-MS: 393.2240 [M+Na]$^+$ ($C_{20}H_{34}O_6Na$, Calcd 393.2253); ^{1}H and ^{13}C NMR data see Tables 1 and 2.

3.6.12. Crystallographic data of compound 7. The structure of **7** was unambiguously determined by single-crystal X-ray diffraction technique. A suitable crystal of compound **7** was obtained by recrystallization from $CHCl_3$ and MeOH (10:1). A colorless crystal with dimensions 0.4 × 0.3 × 0.2 mm^3 was selected for the crystallographic measurements. $C_{20}H_{34}O_6$: M_r 370.47, tetragonal, $a = 12.028(2)$, $b = 12.028(2)$, $c = 25.295(5)$ Å, $\beta = 90°$, $V = 3659.5(11)$ Å^3, space group = $P4_3$, $Z = 8$, $D_{calc} = 1.345$ mg/m^3, $F(0\ 0\ 0) = 1616$, Mo-Kλ = 0.71073 Å. Unit cell dimensions were determined by least-squares fit of 6724 reflections measured at 293(2) K. The intensity data within θ range of 1.69–25.60° were collected at 173.2(2) K and the completeness to θ (25.60°) is 98.8%. A total of 25,208 reflections were collected, of which 6724 reflections were judged observed on the basis of $I > 2\sigma(I)$. The structure was solved by the direct methods and expanded using Fourier transformation techniques and refined by a full-matrix least-squares calculation on F^2 with the aid of program SHELXL97.[26] The final R and R_w factors were measured as 0.0797 and 0.1777, respectively. The figure was plotted with the aid of ORTEPII program.[27] Crystallographic data for compound **7** was deposited in Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

3.6.13. Craiobiotoxin VIII (8). IR (KBr) ν_{max} (cm^{-1}): 3455 (OH), 1625, 1380, 1257, 1040. ESI-MS: *m*/*z* 375 [M+Na]$^+$, 351 [M−H]$^+$, HRESI-MS: 375.2160 [M+Na]$^+$ ($C_{20}H_{32}O_5Na$, Calcd 375.2147); ^{1}H and ^{13}C NMR data see Tables 1 and 2.

3.6.14. Craiobioside A (9). mp: 182–184 °C; IR (KBr) ν_{max} (cm^{-1}): 3435 (OH), 2952, 1380, 1250, 1105, 1045. ESI-MS: *m/z* 555 $[M+Na]^+$, 531 $[M-H]^+$, HRESI-MS: *m/z* 555.2780 $[M+Na]^+$ ($C_{26}H_{44}O_{11}Na$ calc. 555.2781); ^1H and ^{13}C NMR data see Tables 1 and 2.

3.6.15. Craiobioside B (10). mp: 172–174 °C;IR (KBr) ν_{max} (cm^{-1}): 3500 (OH), 2966, 1624, 1378, 1250, 1100, 1045. ESI-MS: *m/z* 537 $[M+Na]^+$, 553 [M+K], 513 $[M-H]^+$, HRESI-MS: *m/z* 537.2665 $[M+Na]^+$ ($C_{26}H_{42}O_{10}Na$, Calcd 537.2676), 553.2428 $[M+K]^+$ ($C_{26}H_{42}O_{10}K$, Calcd 553.2415); ^1H and ^{13}C NMR data see Tables 1 and 2.

Acknowledgments

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