Limonoids from the Seeds of a Hainan Mangrove, *Xylocarpus granatum*

Jian-Yu Pan,[†] Shi-Lin Chen,[†] Min-Yi Li,[‡,§] Jun Li,[‡,§] Mei-Hua Yang,*[,†] and Jun Wu*[,‡]

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, People's Republic of China, Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, People's Republic of China, and Graduate University of Chinese Academy of Sciences, 19 Yuquan Road, Beijing 100049, People's Republic of China

Received June 14, 2010

Ten new limonoids, hainangranatumins A−J (**1**−**10**), and 25 known compounds were isolated from seeds of a Chinese mangrove, *Xylocarpus granatum*, collected on Hainan Island. Hainangranatumins A−E (**1**−**5**) and I and J (**9** and **10**) are 9,10-*seco*-mexicanolides, whereas hainangranatumin F (**6**) is a limonoid possessing an 8α,30α-epoxy ring and a C_1-C_{29} oxygen bridge. Hainangranatumin G (**7**) is a limonoid with a central pyridine ring, and hainangranatumin H (**8**) is a phragmalin 1,8,9-*ortho* ester. The relative configurations of hainangranatumins A and B were established by means of single-crystal X-ray diffraction analysis, and their absolute configurations were assigned on the basis of the specific rotation of the free acids obtained from alkaline hydrolysis. This is the first report of X-ray crystallographic structures of 9,10-*seco*-mexicanolides with a flexible $C_2-C_{30}-C_8$ linkage. Hainangranatumins I and J (**9** and **10**), unusual 9,10-*seco*-mexicanolides with a C_9-C_{30} linkage, are proposed to be artifacts derived from hainangranatumin C and xylomexicanin A, respectively.

Limonoids, highly oxidized triterpene derivatives from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton, have been found only in plants of the order Rutales. Two meliaceous mangroves, *Xylocarpus granatum* and *X. moluccensis*, are known for producing antifeedant limonoids, especially mexicanolides and phragmalins. Gedunin, the first limonoid from plants of the *Xylocarpus* genus, was obtained by Taylor in 1965 from the timber of an African *X. granatum*. To date, more than 120 limonoids have been isolated and identified from the plants of this genus.[1−10]

Extracts of *X. granatum* have traditionally been utilized as astringents and emollients for the treatment of diarrhea, cholera, and fever. The MeOH extract of its bark exhibited significant and dose-dependent antidiarrheal activity, and the aqueous extract of its seeds showed strong antifilarial activity against adult worms of subperiodic *Brugia malayi*.[11] Previously, more than 90 limonoids have been identified from the stem bark, timber, fruits, and seeds of *X. granatum*.[1−10] Herein, we report the isolation and identification of 10 new limonoids including seven 9,10-*seco*-mexicanolides (**1**−**5**, **9**−**10**), a limonoid possessing an 8α,30α-epoxy ring and a C_1-C_{29} oxygen bridge (**6**), a limonoid with a central pyridine ring (**7**), and a phragmalin 1,8,9-*ortho* ester (**8**). All compounds were isolated from the seeds of a Chinese *X. granatum* collected on Hainan Island. In addition to these new compounds, 25 known ones, 6-acetylmexicanolide,[12] 6-acetylxylocarpin,[13] 6-deoxy-swietenine,[14] 6-hydroxymexicanolide,[15] 7-oxogedunin,[16] angustidienolide,[17] azadiradione,[18] granaxylocarpins A and B,[19] methyl 6-hydroxyangolensate,[20] methyl angolensate,[16] mexicanolide,[21] piscidirol G,[22] protoxylocarpins A and D,[6] sapelin E acetate,[23] tigloylseneganolide A,[24] xylocarpin,[25] xylocarpin I,[26] xyloccensins P and Q,[27,28] and xylogranatins C, D,[29] N, and O,[30] were also obtained. The structures of all these compounds were established on the basis of NMR spectroscopic and mass spectrometric data. The relative configurations of compounds **1** and **2** were confirmed by means of single-crystal X-ray diffraction analysis, and their absolute configurations were established on the basis of the specific rotation of the free acids obtained from alkaline hydrolysis.

Results and Discussion

Hainangranatumin A (**1**) was isolated as colorless crystals. The molecular formula $C_{32}H_{40}O_{10}$ was established by ESIMS (*m*/*z* 607.1 $[M + Na]^+$) and NMR data, indicating that **1** had 13 degrees of unsaturation. The ^1H and ^{13}C NMR data (Tables 1 and 2) of **1** indicated that nine of the 13 elements of unsaturation came from two carbonyl groups, three ester functionalities, and four carbon−carbon double bonds. Therefore the molecule has to be tetracyclic. The ^{13}C NMR data with DEPT multiplicity editing experiments revealed that **1** had seven methyl (a methoxy, a primary methyl, two secondary methyl groups, and three tertiary methyl groups), four methylene, 10 methine (five olefinic), and 11 quaternary carbons (five carbonyls).

The NMR data of **1** and its 2D NMR studies ($^1H-^1H$ COSY, HSQC, HMBC, and NOESY) (Figure 1a and b) indicated the presence of a methoxycarbonyl group [δ_H 3.65 s; δ_C 52.0 CH_3; 173.4 qC], a 2-methylbutyryl group [δ_H 0.84 (t, J = 7.5 Hz), 1.13 (d, J = 7.0 Hz), 1.41 m, 1.66 m, 2.37 m; δ_C 11.9 CH_3, 17.1 CH_3, 26.1 CH_2, 41.3 CH, 175.2 qC], a β-furanyl ring [δ_H 7.52 br s, 6.44 br s, and 7.42 br s; δ_C 119.6 qC, 141.4 CH, 109.8 CH, and 143.3 CH], and two carbonyl carbons [δ_C 208.7 qC, 198.8 qC]. An α,β-unsaturated δ-lactone ring D, characterized by the NMR data [δ_H 6.14 s, 5.29 s; δ_C 118.8 CH, 80.2 CH, 38.4 qC, 163.3 qC, and 163.4 qC], was confirmed by HMBC correlations between H-17/C-13, H-17/C-14, H-17/C-16, H-15/C-13, H-15/C-14, and H-15/C-16 (Figure 1a). A proton of an olefinic methine [δ_H 6.94 s; δ_C 161.6 CH] was assigned to H-3 on the basis of its HMBC correlations to C-1 (δ_C 198.8 qC), C-2 (δ_C 128.8 qC), C-4 (δ_C 36.9 qC), C-5 (δ_C 45.2 CH), and C-30 (δ_C 67.0 CH). Therefore the $\Delta^{2,3}$ double bond in ring A of **1** was conjugated with the C-1 carbonyl group. Protons of a secondary methyl group [δ_H 1.00 (br d, J = 4.5 Hz); δ_C 11.5], showing HMBC correlations to C-1, C-5, and C-10 (δ_C 42.8 CH), were identified as H_3-19. These 1D and 2D NMR data strongly suggested that **1** was a 9,10-*seco*-mexicanolide. HMBC correlations from H_2-11 [δ_H 2.50 (m, H-11α), 3.01 (dd, J = 19.1, 6.1 Hz, H-11β)] to the C-9 carbonyl carbon (δ_C 208.7 qC) further confirmed the 9,10-*seco* feature of **1**.

Protons of a tertiary methyl group [δ_H 0.95 s; δ_C 18.6 CH_3], showing HMBC correlations to C-12, C-13, and C-17, were

* To whom correspondence should be addressed. Tel & Fax: (86-10) 6289-9730. E-mail: yangmeihua15@hotmail.com (M.-H.Y.). Tel: (86-20) 8445-8442. Fax: (86-20) 8445-1672. E-mail: wwujun2003@yahoo.com (J.W.).

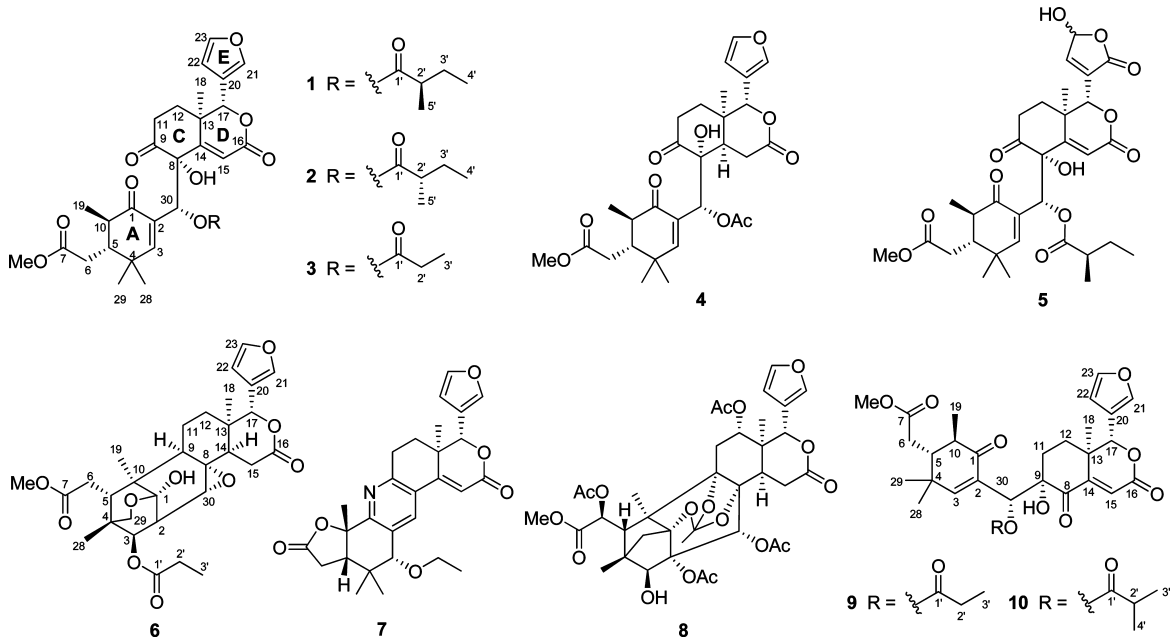
[†] Chinese Academy of Medical Sciences and Peking Union Medical College.

[‡] South China Sea Institute of Oceanology, Chinese Academy of Sciences.

[§] Graduate University of Chinese Academy of Sciences.

10.1021/np100395w
Published on Web 09/15/2010

Chart 1



assigned to H_3-18. Protons of two tertiary methyl groups [δ_H 1.15 s, δ_C 27.9 CH_3; δ_H 1.09 s, δ_C 20.3 CH_3], exhibiting HMBC correlations to C-4, were identified as H_3-28 and H_3-29, respectively. The NMR data of **1** were similar to those of granaxylocarpin B,[18] except for the replacement of the 30-*O*-tigloyl group in granaxylocarpin B by a 2-methylbutyryl group. The HMBC correlation from H-30 (δ_H 6.44 s) of **1** to the carbonyl carbon [δ_C 175.2 qC] of this 2-methylbutyryl group further confirmed the location of this acyl function at C-30. Thus, the planar structure of **1** was identified as a 30-*O*-(2-methylbutanoate) analogue of granaxylocarpin B.

The relative configuration of **1** was partially established on the basis of NOE interactions. Significant NOE interactions (Figure 1b) between H-12β/H-17 and H-11α/H_3-18 helped to establish the α-orientation of H_3-18 and β-orientation of H-17. However, the orientation of H-5 and H-10 in ring A could not be assigned by NOE interactions due to their overlapped signals in the ^{1}H NMR spectrum. Moreover, the orientation of 8-OH was difficult to establish without essential NOE interactions.

In order to establish the relative configuration of **1**, single-crystal X-ray diffraction analysis was employed. A computer-generated perspective drawing of the X-ray model of **1** is given in Figure 1c. Compound **1** is a 9,10-*seco*-mexicanolide with $\Delta^{2,3}$ and $\Delta^{14,15}$ double bonds. A 2-methylbutanoyloxy group is located at C-30 in an α-orientation. The orientation of H-5, H-30, and H_3-19 is β, and that of H-10, H-2′, and 8-OH is α. Three six-membered rings in its crystal structure, viz., A, C, and D, adopt half-chair conformations, whereas the furan ring E is planar.

The α_D value is negative for (*R*)-2-methylbutyric acid (−14.3)[31] and positive for (*S*)-2-methylbutyric acid (+19.3, 18.9).[32,33] Therefore the absolute configuration at C-2 in the 2-methylbutyryl group of **1** can be determined according to the specific rotation of its acid, which was obtained from the alkaline hydrolysis of **1**. The absolute configuration at C-2′ was suggested as *R* by the negative α_D value {$[\alpha]^{25}_D$ −6.7 (*c* 0.03, Me_2CO)} of the 2-methylbutyric acid. On the basis of the aforementioned relative configuration of **1** from single-crystal X-ray diffraction analysis, the other six stereogenic centers at C-5, C-8, C-10, C-13, C-17, and C-30 in **1** were assigned as *R* (Figure 1c). Therefore, the full structure of **1**, named hainangranatumin A, including the absolute configuration, was unambiguously established as shown.

Hainangranatumin B (**2**), colorless crystals, had the same molecular formula as that of **1**. The NMR data of **2**, indicating the presence of a 2-methylbutyryl group, were similar to those of **1**. However, the resonances of H_3-5′ [δ_H 1.10 (d, J = 7.0 Hz)] of the 2-methylbutyryl group in **2** were slightly upfield shifted. This observation disclosed the different absolute configuration at C-2 [δ_H 2.42 m; δ_C 40.9 CH] of this group. HSQC, HMBC, and NOESY experiments revealed that **2** was a 9,10-*seco*-mexicanolide possessing the same skeleton as that of **1**.

Single-crystal X-ray diffraction analysis permitted the establishment of the relative configuration of **2**. A computer-generated perspective drawing of the X-ray model of **2** is given in Figure 1d. Four rings, designated as A, C, D, and E, were found in **2**, and their conformations in the crystal were the same as those of **1**. However, the orientation of H-2 of the 2-methylbutyryl group is opposite that of the same group in **1**. Alkaline hydrolysis of **2** yielded a corresponding 2-methylbutanoic acid. The absolute configuration of its C-2 was concluded to be *S* from its positive α_D value {$[\alpha]$ = +10.0 (*c* 0.04, Me_2CO)}.[32,33] On the basis of the relative configuration of **2** from single-crystal X-ray diffraction analysis, the absolute configurations at C-5, C-8, C-10, C-13, C-17, and C-30 in **2** were assigned as *R* (Figure 1d). Therefore, the full structure of **2**, named hainangranatumin B, including the absolute configuration, was established as shown.

Hainangranatumin C (**3**), colorless crystals, had the molecular formula $C_{30}H_{36}O_{10}$, as established by HR-TOFMS (*m*/*z* 579.2185, calcd for $[M + Na]^+$ 579.2206). The NMR data of **3** were similar to those of **1** (Tables 1 and 2), except for the replacement of the 30-*O*-2′(*R*)-methylbutyryl group in **1** by a propionyl group [δ_H 1.11 (t, J = 7.5 Hz), 2.35 m; δ_C 173.4 qC, 27.7 CH_2, 9.0 CH_3]. The substructure of the propionyl group was confirmed by HMBC cross-peaks between H_3-3′/C-2′, H_2-2′/C-1′, and H_2-2′/C-3′ and ^{1}H−^{1}H COSY correlations between H_3-3′/H_2-2′. The HMBC cross-peak from H-30 (δ_H 6.50 s) to the carbonyl carbon (δ_C 173.4) of the propionyl group assigned its location at C-30. Thus, the structure of **3**, named hainangranatumin C, was established as shown.

Hainangranatumin D (**4**) was obtained as a white, amorphous powder. The molecular formula $C_{29}H_{36}O_{10}$ was established by HR-TOFMS (*m*/*z* 567.2173, calcd for $[M + Na]^+$ 567.2206; *m*/*z* 545.2371, calcd for $[M + H]^+$ 545.2387), indicating 12 degrees of unsaturation. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **4** indicated that eight of the 12 elements of unsaturation came from two carbonyl groups, three carbon−carbon double bonds, and three ester functionalities. Therefore the molecule was tetracyclic. The NMR data of **4** were similar to those of **1**, except for the presence of an acetoxy group and the absence of a $\Delta^{14,15}$ double bond and a

Table 1. ^{1}H (500 MHz) NMR Data (δ) for Compounds **1**−**5** in $CDCl_3$ (J in Hz)

position	**1**	**2**	**3**	**4**	**5**
3	6.94 s	6.97 s	6.97 s	6.80 s	6.94 s
5	2.24[a]	2.26[a]	2.26[a]	2.24[a]	2.24[a]
6a	2.24[a]	2.26[a]	2.26[a]	2.24[a]	2.28 m
6b	2.48 m	2.47 m	2.44 m	2.46 m	2.46 m
10	2.24[a]	2.26[a]	2.26[a]	2.24[a]	2.24[a]
11α	2.50 m	2.52 m	2.51 m	1.72 m	2.55 m
11β	3.01 dd (19.1, 6.1)	3.04 dd (19.5, 6.5)	3.01 dd (19.7, 6.6)	2.33 m	3.12 dd (19.8, 6.8)
12α	1.62 m	1.63[a]	1.62 dd (13.6, 7.1)	2.52 m	1.65 m
12β	2.56 m	2.60 m	2.60 m	2.67 ddd (18.4, 6.1, 6.1)	2.88 m
14				2.13 br d (8.0)	
15α	6.14 s	6.15 s	6.13 s	2.77 dd (17.5, 8.0)	6.17 s
15β				3.11 dd (17.5, 7.5)	
17	5.29 s	5.31 s	5.31 s	5.36 s	5.34/5.31 s[b]
18	0.95 s	0.96 s	0.95 s	1.06[a]	1.01/0.99 s[b]
19	1.00 br d (4.5)	1.02 br d (4.5)	1.01 d (5.8)	1.06[a]	1.03 d (4.2)
21	7.52 br s	7.54 br s	7.53 br s	7.52 s	
22	6.44 br s	6.45 br s	6.44 br s	6.45 br s	7.43/7.40 br s[b]
23	7.42 br s	7.43 br s	7.42 br s	7.43 br s	6.24 br s
28	1.15 s	1.16 s	1.17 s	1.14 m[a]	1.17 s
29	1.09 s	1.11 s	1.09 s	1.06 s	1.11 s
30	6.44 s	6.47 s	6.50 s	6.30 s	6.42 s
7-OMe	3.65 s	3.67 s	3.66 s	3.68 s	3.69 s
8-OH	3.89 s	3.88 s	3.88 s	4.38 s	3.93/3.92 s[b]
30-Acyl					
2′	2.37 m	2.42 m	2.35 m	2.10 s	2.39 m
3′a	1.41 m	1.50 m	1.11 t (7.5)		1.42 m
3′b	1.66 m	1.63[a]			1.70 m
4′	0.84 t (7.5)	0.86 t (7.5)			0.85 t (7.5)/0.86 t (7.5)[b]
5′	1.13 d (7.0)	1.10 d (7.0)			1.14 d (7.5)

[a] Overlapped signals assigned by HSQC and HMBC spectra without designating multiplicity. [b] Pairs of signals resulted from C-23 epimers.

Table 2. ^{13}C (125 MHz for **1** and **2** and 100 MHz for **3**−**5**) NMR Data (δ) for Compounds **1**−**5** in $CDCl_3$

position	**1**	**2**	**3**	**4**	**5**
1	198.8 qC	198.8 qC	198.7 qC	199.7 qC	198.6 qC
2	128.8 qC	128.7 qC	128.6 qC	131.0 qC	128.84/128.79 qC[b]
3	161.6 CH	161.7 CH	161.9 CH	160.4 CH	161.50/161.37 CH[b]
4	36.9 qC	36.9 qC	36.8 qC	36.7 qC	36.9 qC
5	45.2 CH	45.2 CH	45.2 CH	45.0 CH	45.2 CH
6	34.6 CH_2	34.6 CH_2	34.6 CH_2	34.7 CH_2	34.7 CH_2
7	173.4 qC	173.4 qC	173.4 qC[a]	173.4 qC	173.4 qC
8	80.1 qC	80.1 qC	80.1 qC[a]	79.0 qC	80.2 qC
9	208.7 qC	208.6 qC	208.6 qC	210.7 qC	208.3 qC
10	42.8 CH	42.8 CH	42.8 CH	43.1 CH	42.8 CH
11	33.0 CH_2	33.0 CH_2	33.0 CH_2	29.9 CH_2	33.0 CH_2
12	25.7 CH_2	25.6 CH_2	25.5 CH_2	33.6 CH_2	25.9 CH_2
13	38.4 qC	38.3 qC	38.3 qC	36.9 qC	38.57/38.47 qC[b]
14	163.3 qC	163.3 qC	164.0 qC	48.3 CH	162.46/162.37 qC[b]
15	118.8 CH	118.6 CH	118.2 CH	28.7 CH_2	118.5 CH
16	163.4 qC	163.5 qC	163.2 qC	170.9 qC	163.6 qC
17	80.2 CH	80.2 CH	80.1 CH[a]	78.4 CH	78.25/78.02 CH[b]
18	18.6 CH_3	18.6 CH_3	18.5 CH_3	22.9 CH_3	18.57/18.49 CH_3[b]
19	11.5 CH_3	11.5 CH_3	11.6 CH_3	11.7 CH_3	11.6 CH_3
20	119.6 qC	119.6 qC	119.6 qC	120.2 qC	133.21/133.13 qC[b]
21	141.4 CH	141.4 CH	141.4 CH	141.1 CH	168.85/168.56 qC[b]
22	109.8 CH	109.8 CH	109.8 CH	109.8 CH	150.17/149.52 CH[b]
23	143.3 CH	143.2 CH	143.2 CH	143.2 CH	97.19/96.50 CH[b]
28	27.9 CH_3	27.9 CH_3	27.8 CH_3	27.8 CH_3	28.0 CH_3
29	20.3 CH_3	20.3 CH_3	20.5 CH_3	20.2 CH_3	20.4 CH_3
30	67.0 CH	67.0 CH	67.1 CH	70.3 CH	67.5 CH
7-OMe	52.0 CH_3	52.0 CH_3	51.9 CH_3	52.0 CH_3	52.0 CH_3
30-Acyl					
1′	175.2 qC	175.7 qC	173.4 qC[a]	169.4 qC	175.04/174.95 qC[b]
2′	41.3 CH	40.9 CH	27.7 CH_2	21.3 CH_3	41.3 CH
3′	26.1 CH_2	27.1 CH_2	9.0 CH_3		26.2 CH_2
4′	17.1 CH_3	16.3 CH_3			17.1 CH_3
5′	11.9 CH_3	11.7 CH_3			11.9 CH_3

[a] Overlapped signals. [b] Pairs of signals resulted from C-23 epimers.

30-*O*-2-methylbutyryl group. It was suggested that the 30-*O*-2-methylbutyryl group in **1** was replaced by a 30-acetoxy group in **4**. The appearance of an aliphatic methine group [δ_H 2.13 (br d, J = 8.0 Hz, H-14); δ_C 48.3] and an aliphatic methylene group [2.77 (dd, J = 17.5, 8.0 Hz, H-15α), 3.11 (dd, J = 17.5, 7.5 Hz, H-15β); δ_C 28.7] and the downfield shift of C-16 (δ_C 170.9) in **4** suggested the existence of the substructure CH-14−CH_2-15, which was further confirmed by HMBC cross-peaks between H_2-15/C-13, H_2-15/C-14, H_2-15/C-16, H-14/C-8, H-14/C-13, H-14/C-15, and H-14/C-16. The HMBC cross-peak from H-30 (δ_H 6.30 s) to the carbonyl

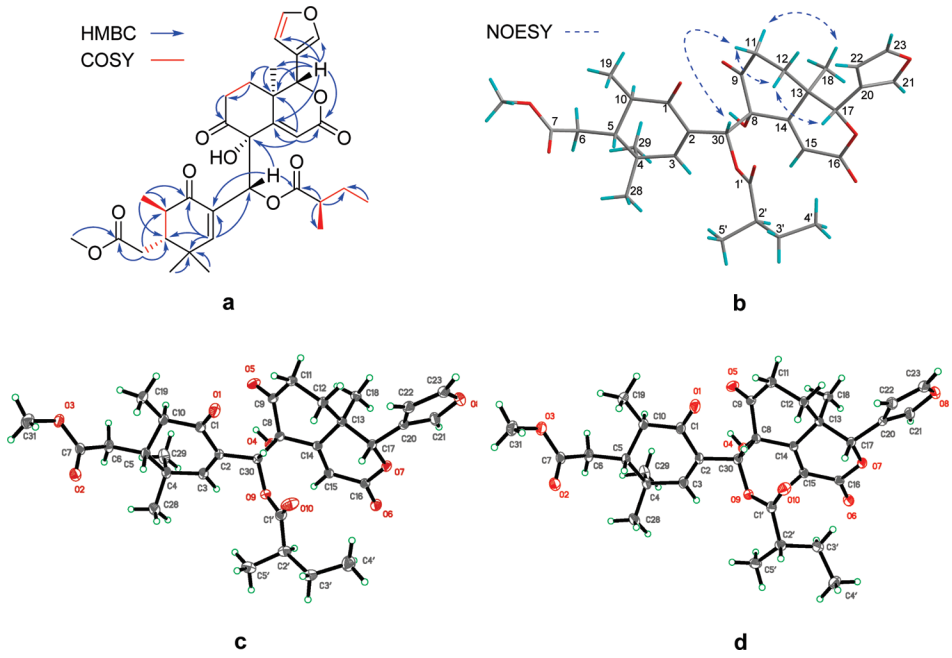


Figure 1. (a) Selected ^{1}H−^{1}H COSY and HMBC correlations for hainangranatumin A (**1**); (b) key NOE correlations for **1**; (c) perspective drawing of the X-ray structure for **1**; (d) perspective drawing of the X-ray structure for hainangranatumin B (**2**).

carbon (δ_C 169.4) of the acetoxy group assigned its location at C-30. Thus, the structure of **4**, named hainangranatumin D, was established as shown.

Hainangranatumin E (**5**), a white, amorphous powder, had the molecular formula $C_{33}H_{42}O_{12}$, as established by HR-TOFMS (*m/z* 639.2435, calcd for $[M + Na]^+$ 639.2417). Its ^{1}H and ^{13}C NMR data (Tables 1 and 2) were similar to those of **1**, except for the replacement of the β-furanyl ring in **1** by a γ-hydroxybutenolide group, which was characterized by pairs of protons at δ_H 7.43/7.40 (H-22), 5.34/5.31 (H-17), 3.93/3.92 (8-OH), and 1.01/0.99 (H_3-18) and by pairs of carbons at δ_C 133.21/133.13 (C-20), 168.85/168.56 (C-21), 150.17/149.52 (C-22), and 97.19/96.50 (C-23). The γ-hydroxybutenolide group in **5** was the same as that in moluccensin N[5] and domesticulide.[15] HMBC cross-peaks between H-17/C-20 and H-17/C-22 revealed the linkage of the γ-hydroxybutenolide group to C-17. Moreover, carbon resonances at δ 38.57/38.47 (C-13), 162.46/162.37 (C-14), 78.25/78.02 (C-17), 18.57/18.49 (C-18), 128.84/128.79 (C-2), 161.50/161.37 (C-3), and 175.04/174.95 (C-1′) resonated as pairs. The appearance of pairs of the above proton and carbon resonances in the NMR spectra of **5** suggested the presence of C-23 epimers. Significant NOE interactions between H-11β/H-30, H-12β/H-30, H-17/H-12β, and H-11α/H_3-18 helped to establish the β-orientation of H-17 and the α-orientation of H_3-18. Therefore, the structure of **5**, named hainangranatumin E, was established as shown.

Hainangranatumin F (**6**), a white, amorphous powder, had the molecular formula $C_{30}H_{38}O_{10}$, as established by HR-TOFMS (*m/z* 581.2319, calcd for $[M + Na]^+$ 581.2363; *m/z* 559.2549, calcd for $[M + H]^+$ 559.2543). Its NMR data (Table 3) were similar to those of xyloccensin L,[34] which is a highly oxidized limonoid with an 8α,30α-epoxy ring and a C_1−C_{29} oxygen bridge, isolated from the same plant, except for the replacement of the 3-*O*-tigloyl group in xyloccensin L by a propionyl group [δ_H 2.55 (q, J = 7.5 Hz), 1.21 (t, J = 7.5 Hz); δ_C 173.9, qC, 27.5 CH_2, 9.2 CH_3]. The strong HMBC cross-peak from H-3 [δ_H 5.31, (d, J = 10.4 Hz)] to the carbonyl carbon (δ_C 173.9, qC) of the propionyl group confirmed its location at C-3 (Figure 2a). Moreover, NOE interactions observed in **6** from H-5 to H_3-28 and H-30 concluded the β-orientation of H-5 and H-30. Similarly, NOE interactions between H-17/H-12β and H-17/H-15β indicated the β-orientation of H-17, and those between H-14/H-15α, H-14/H_3-18, and H-9/H_3-18 established the α-orientation of H-9, H-14, and H_3-18 (Figure 2b). Hence, the structure of compound **6**, named hainangranatumin F, was established as shown.

Hainangranatumin G (**7**), a colorless, amorphous powder, had the molecular formula $C_{28}H_{31}NO_6$, as established by HR-TOFMS (*m/z* 478.2239, calcd for $[M + H]^+$ 478.2230). The ^{1}H and ^{13}C NMR data (Table 3) of **7** were similar to those of xylogranatin G,[30] which is a B,C-*seco*-limonoid with a central pyridine ring, except for the replacement of the 3-acetoxy group in xylogranatin G by an ethoxy group [δ_H 3.40 m, 1.15 (t, J = 7.0 Hz); δ_C 64.5 CH_2, 15.2 CH_3]. HMBC correlations between H_2-1′/C-2′ and H_3-2′/C-1′ confirmed the substructure of the ethoxy group, and those between H-3/C-1′ and H_2-1′/C-3 assigned its location at C-3. The relative configuration of **7** was found to be the same as that for xylogranatin G[30] by NOE interactions. Thus, the structure of compound **7**, named hainangranatumin G, was established as shown.

Hainangranatumin H (**8**), a colorless, amorphous powder, had the molecular formula $C_{37}H_{44}O_{17}$, as established by HR-TOFMS (*m/z* 783.2464, calcd for $[M + Na]^+$ 783.2476; *m/z* 761.2676, calcd for $[M + H]^+$ 761.2657). Its ^{1}H and ^{13}C NMR data (Table 4) indicated that **8** was a phragmalin *ortho*-ester, characterized by a methyl singlet at δ_H 1.66 and its HMBC correlation to a quaternary carbon at δ_C 119.2. Three oxygenated quaternary carbons at δ_C 85.1, 85.6, and 86.0 further supported the identification of this *ortho*-ester. Moreover, the presence of four acetoxy groups [δ_H 1.63, δ_C 169.2 qC, 20.1 CH_3; δ_H 2.19, δ_C 169.7 qC, 21.4 CH_3; δ_H 2.21, δ_C 170.9 qC, 21.3 CH_3; δ_H 2.09, δ_C 169.1 qC, 21.2 CH_3] was indicated from the NMR data of **8**. HMBC correlations from H-6, H-12, and H-30 to the carbonyl carbons of three acetoxy groups at δ_C 169.7 qC, 170.9 qC, and 169.1 qC, respectively, assigned their location at C-6, C-12, and C-30 of the phragmalin nucleus (Figure 2c). A proton at δ_H 2.70 (1H, br s), which was not correlated with a carbon signal in the HSQC spectrum, indicated the presence of a hydroxy group in **8**. HMBC correlations from this proton to C-2 and C-3 suggested the location of the hydroxy group at C-2 or C-3. HMBC correlations from H-3 to C-2 and C-4, but not from H-3 to the carbonyl carbon at δ_C 169.2 qC, suggested the location of the hydroxy group at C-3 and the fourth acetoxy group at C-2. The upfield shift of C-3 (δ_C 83.9 CH) and the downfield shift of C-2 (δ_C 79.7 qC) in comparison with those of xyloccensin S[35] further confirmed the above assignments. NOE interactions between H-15β/H-17,

Table 3. ^{1}H (500 MHz) and ^{13}C (100 MHz) NMR Data (δ) for Compounds **6** and **7** in $CDCl_3$

	6		**7**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		97.0 qC		157.8 qC
2	2.99 dd (10.4, 2.3)	41.4 CH		128.5 qC
3	5.31 d (10.4)	74.7 CH	3.82 s	83.8 CH
4		37.1 qC		36.1 qC
5	2.74 br d (10.8)	35.3 CH	2.98[a]	45.0 CH
6a	2.30 br d (16.5)	31.9 CH_2	2.55 m	30.6 CH_2
6b	2.40 dd (16.5, 10.8)		2.98[a]	
7		173.92 qC		175.5 qC
8		61.3 qC		123.3 qC
9	2.07 dd (13.5, 4.8)	47.0 CH		158.0 qC
10		41.4 qC		84.1 qC
11α	1.84 m	18.5 CH_2	3.12 m	27.9 CH_2
11β	1.74 td (13.6, 3.2)		3.19 m	
12α	1.37 td (13.6, 3.2)	33.7 CH_2	1.87 ddd (13.0, 5.1, 1.9)	30.2 CH_2
12β	2.01 td (13.6, 3.2)		1.77 m	
13		36.0 qC		37.6 qC
14	1.70 br d (5.5)	45.7 CH		157.3 qC
15α	2.79 dd (16.8, 5.5)	32.4 CH_2	6.54 s	111.0 CH
15β	3.11 dd (16.8, 11.7)			
16		171.3 qC		165.1 qC
17	5.24 s	79.3 CH	5.23 s	81.0 CH
18	1.04 s	26.0 CH_3	1.17 s	15.8 CH_3
19	1.05 s	14.8 CH_3	1.76 s	28.5 CH_3
20		120.4 qC		119.8 qC
21	7.54 s	141.0 CH	7.55 br s	141.4 CH
22	6.44 br s	110.1 CH	6.52 br s	110.0 CH
23	7.43 br s	143.2 CH	7.48 br s	143.3 CH
28	0.60 s	14.9 CH_3	1.15 s	24.1 CH_3
29$_{pro\text{-}S}$	3.89 d (8.0)	67.5 CH_2	0.67 s	20.4 CH_3
29$_{pro\text{-}R}$	3.53 d (8.0)			
30	3.18 d (2.3)	60.4 CH	7.79 s	133.7 CH
7-OMe	3.70 s	52.2 CH_3		
1-OH	3.76 s			
	3-Propionyl		3-Ethoxy	
1′		173.9 qC	3.40 m	64.5 CH_2
2′	2.55 q (7.5)	27.5 CH_2	1.15 t (7.0)	15.2 CH_3
3′	1.21 t (7.5)	9.2 CH_3		

[a] Overlapped signals assigned by HSQC and HMBC spectra without designating multiplicity.

H-15β/H-30, H-17/H-12, and H-17/H-30 established the β-orientation of H-12, H-17, and H-30. Similarly, those between H-14/H_3-18 and H-14/H-15α indicated the α-orientation of H-14 and H_3-18 (Figure 2d), and those between H-30/H-5 and H-5/H_3-28 indicated the β-orientation of H-5 and H_3-28. The NOE interaction from H-3 to $H_{pro\text{-}R}$-29, but not from H-3 to H-5, H_3-28, and H-30, suggested the α-orientation of H-3. Thus, the structure of compound **8**, named hainangranatumin H, was established as shown.

Hainangranatumin I (**9**) had the same molecular formula as that of **3**, viz., $C_{30}H_{36}O_{10}$, as established by HR-TOFMS (*m*/*z* 579.2239, calcd for $[M + Na]^+$ 579.2206). It indicated that **9** might be an isomer of **3**. Comparison of its ^{1}H and ^{13}C NMR data (Table 5) with those of **3** revealed that structures of both compounds were indeed closely related. However, the main differences occurred at C-8 and C-9. HMBC correlations between H_2-11/C-30 (δ_C 66.3 CH), H-15/C-8 (δ_C 200.8 qC), and H_2-11/C-9 (δ_C 79.7 qC) (Figure 2e) suggested the linkage of the C-30 methine to C-9, which led to the formation of a hydroxylated quaternary carbon at C-9 and a conjugated carbonyl group at C-8. The above linkage pattern was further confirmed by the remarkable upfield shifts of H_2-11 [δ_H 1.79 (ddd, J = 14.6, 14.5, 4.0 Hz, 11α), 1.67 (H, ddd, J = 14.6, 3.6, 3.6 Hz, 11β) in **9**; δ_H 2.51 (m, 11α), 3.01 (dd, J = 19.7, 6.6 Hz, 11β) in **3**] and the UV red shift of the molecule (UV λ_{max} 240.7 nm in **9** and λ_{max} 212.5 nm in **3**).

The relative configuration of **9** was determined by NOE interactions as shown in Figure 2f. The NOE interaction between H-12α/H_3-18 indicated the α-orientation of H_3-18, whereas NOE interactions between H-30/H-17, H-30/12β, and H-30/11β indicated the β-orientation of H-17 and H-30. Similarly, those between H-5/H_3-19 and H-5/H_3-28 proved the β-orientation of H-5 and H_3-19. Thus, the structure of compound **9**, named hainangranatumin I, was established as shown.

Hainangranatumin J (**10**) had the molecular formula $C_{31}H_{38}O_{10}$, as established by HR-TOFMS (*m*/*z* 593.2352, calcd for $[M + Na]^+$ 593.2363). The NMR data of **10** were similar to those of **9** (Table 5), except for the replacement of the 30-*O*-propionyl moiety in **9** by an isobutyryl group [δ_H 2.46 m, 1.11 (d, J = 7.0 Hz), 1.09 (d, J = 7.0 Hz); δ_C 174.8 qC, 33.8 CH, 18.8 CH_3, 18.8 CH_3]. The substructure of the isobutyryl group was confirmed by HMBC cross-peaks between H-2′/C-1′, H-2′/C-3′, and H-2′/C-4′ and ^{1}H−^{1}H COSY correlations between H-2′/H_3-3′ and H-2′/H_3-4′. The HMBC cross-peak from H-30 (δ_H 6.21, s) to the carbonyl carbon (δ_C 174.8) of the isobutyryl group assigned its location at C-30. Thus, the structure of compound **10**, named hainangranatumin J, was established as shown.

The first limonoid with a C_{30}−C_9 linkage was xylogranatin D, which was reported as a genuine natural product from seeds of a Chinese mangove, *X. granatum*.[29] However, it was later found to be an artifact derived from xylogranatin C, a 9,10-*seco*-mexicanolide with a C_{30}−C_8 linkage. Long-time storage of xylogranatin C in MeOH could yield xylogranatin D.[30] Hainangranatumins I (**9**) and J (**10**) are the other two limonoids with a C_{30}−C_9 linkage. Similarly, they could be proposed to be artifacts derived from hainangranatumin C (**3**) and xylomexicanin A,[8] respectively, though the latter was not obtained in our experiment.

To date, ten 9,10-*seco*-mexicanolides have been reported. They have been identified from seeds of the Chinese mangrove, *X.*

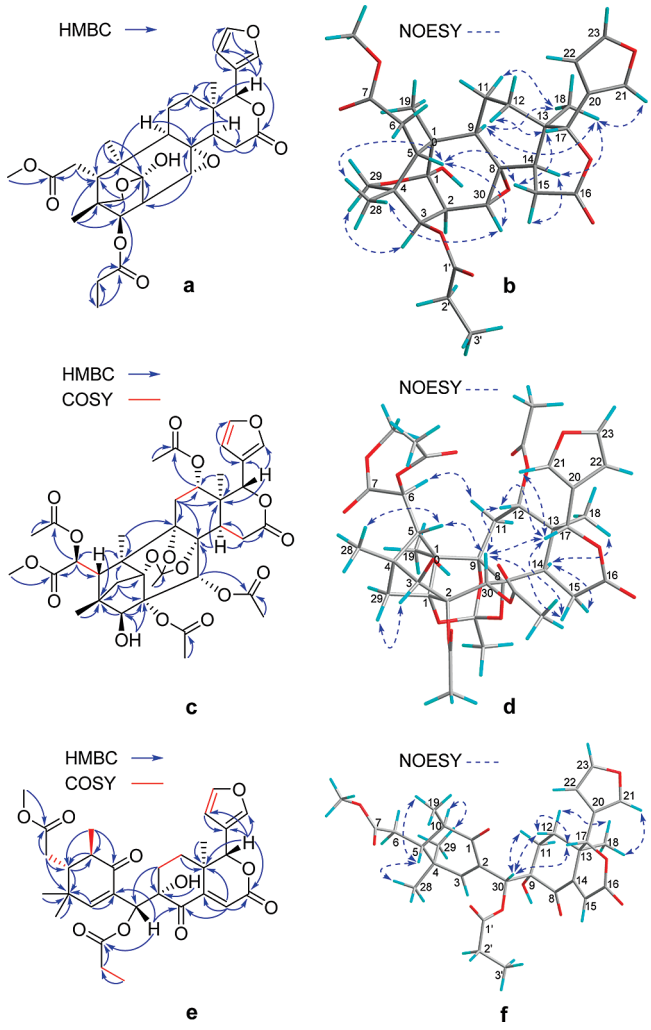


Figure 2. (a) Selected HMBC correlations for hainangranatumin F (**6**); (b) key NOE correlations for **6**; (c) selected ^{1}H−^{1}H COSY and HMBC correlations for hainangranatumin H (**8**); (d) key NOE correlations for **8**; (e) selected ^{1}H−^{1}H COSY and HMBC correlations for hainangranatumin I (**9**); (f) key NOE correlations for **9**.

granatum,[8,10,19,26,29] and can be classified into two substructural classes, of which one possesses a 1,9-oxygen bridge to form a pyran ring, while the other one contains a flexible C_2−C_{30}−C_8 linkage. Before this study, xylogranatin A, the structure of which was supported by X-ray crystallographic analysis,[29] has been the only example belonging to the former class. Previously, the relative configuration of ring A in 9,10-*seco*-mexicanolides of the latter class has been derived from NOE data. However, the orientation of H-5 and H-10 in the A ring was difficult to assign due to their overlapped signals with one signal of H_2-6. In this paper, single-crystal X-ray diffraction analysis was successfully employed to establish the relative configurations of two 9,10-*seco*-mexicanolides with a flexible C_2−C_{30}−C_8 linkage, viz., hainangranatumins A and B (**1** and **2**).

In summary, seven 9,10-*seco*-mexicanolides, named hainangranatumins A−E (**1**−**5**) and I and J (**9** and **10**), were identified from the seeds of a Chinese mangrove, *X. granatum*, collected on Hainan Island, along with a limonoid possessing an 8α,30α-epoxy ring and a C_1−C_{29} oxygen bridge (**6**), a limonoid with a central pyridine ring (**7**), and a phragmalin 1,8,9-*ortho* ester (**8**). Twenty-five known compounds were also obtained. Hainangranatumin A (**1**) was found to possess a 2*R*-methylbutyryl group, whereas hainangranatumin B (**2**) a 2*S*-methylbutyryl group. They are epimers with the same 9,10-*seco*-mexicanolide skeleton. The absolute configurations of hainangranatumins A and B were unequivocally determined on the basis of their X-ray crystallographic structures and the specific rotations of their 2-methylbutyryl groups. This is the first report of X-ray crystallographic structures of 9,10-*seco*-mexicanolides with a flexible C_2−C_{30}−C_8 linkage. In addition, hainangranatumin A is the first limonoid with a 2*R*-methylbutyryl group from plants of the *Xylocarpus* genus. This study also demonstrates that *X. granatum* is a rich source for the production of novel limonoids.

Experimental Section

General Experimental Procedures. Melting points were measured on an X_4 micromelting point detector (Beijing Tech. Instrument Co., Ltd., China). Optical rotations were recorded on a Polaptronic HNQW5 automatic high-resolution polarimeter (Schmidt & Haensch Co., Ltd.). UV spectra were obtained on a Beckman DU-640 UV spectrophotometer, and NMR spectra were recorded in $CDCl_3$ using a Bruker AV-500 or AV-400 spectrometer with TMS as internal standard. HR-TOFMS spectra were measured on a Thermo Scientific LTQ XL spectrometer or Waters Acquity UPLC-Q-Tof Micro MS in positive ion mode. Single-crystal X-ray diffraction analysis was performed on a Bruker Smart 1000 CCD single-crystal diffractometer. Preparative HPLC was carried out on ODS columns (YMC-Pack ODS-5-A, 250 × 10 mm i.d., 5 μm) with a Waters 2998 photodiode array detector. For CC, silica gel (200−300 mesh) (Qingdao Marine Chemical Ind. Co., Ltd.) and RP C_{18} gel (Cosmosil C18-PREP 140 μm, Nacalai Tesque, Kyoto, Japan) were used.

Plant Material. Seeds of *X. granatum* were collected in June 2009 on Hainan Island, China. The identification of the plant was performed by one of the authors (J.W.). A voucher sample (No. HNXG-09-3) is maintained in the herbarium of the South China Sea Institute of Oceanology.

Extraction and Isolation. Dried seeds (20 kg) of *X. granatum* were extracted three times with 95% EtOH at room temperature. The extract was concentrated under reduced pressure, followed by suspension in H_2O and extraction with EtOAc. The EtOAc extract was further suspended in H_2O and extracted with $CHCl_3$. The resulting $CHCl_3$ extract (357 g) was subjected to silica gel CC eluted using a $CHCl_3$−MeOH system (100:0−5:1) to yield 322 fractions. According to the analytic results of thin-layer chromatography, fractions 27 to 62 were combined as portion A (44.5 g) and fractions 100 to 170 were combined as portion B (93.4 g). Portion A was further purified using RP C_{18} CC (MeCN−H_2O, 50:50−100:0) to afford 101 fractions (A1−A101), whereas portion B was purified using silica gel CC to give seven fractions B1−B7. Fraction B7 was purified using RP C_{18} CC (MeCN−H_2O, 30:70−100:0) to afford 95 subfractions (Ba1−Ba95).

Fractions A11 to A14 (1.8 g) from portion A were combined and fractionated by RP C_{18} CC (Me_2CO−H_2O, 30:70−100:0) to afford subfractions Aa1−Aa16. Subfractions Aa9 and Aa10 were combined and purified by preparative HPLC (MeOH−H_2O, 55:45) to give compounds **6** (4.1 mg) and 6-hydroxymexicanolide (3.8 mg). Subfractions Aa12 and Aa13 were combined and purified by preparative HPLC (MeCN−H_2O, 49:51) to yield compounds **4** (3.8 mg), **5** (0.9 mg), **8** (2.4 mg), methyl 6-hydroxyangolensate (2.2 mg), xyloccensins P (58.2 mg) and Q (20.7 mg), and xylogranatins C (14.9 mg) and D (84.4 mg).

Fractions A26 to A35 were combined and fractionated by RP C_{18} CC (MeCN−H_2O, 30:70−100:0) to give subfractions Ab1−Ab32. Subfraction Ab16 was purified by preparative HPLC (MeCN−H_2O, 49:51) to afford compounds **7** (1.0 mg), 7-oxogedunin (1.9 mg), mexicanolide (12.7 mg), 6-acetylmexicanolide (6.4 mg), and 6-acetylxylocarpin (1.6 mg). Subfractions Ab17 and Ab18 were combined and purified by preparative HPLC (MeOH−H_2O, 67:33) to yield compounds **3** (37.9 mg), **9** (2.1 mg), **10** (6.1 mg), angustidienolide (0.8 mg), azadiradione (11.5 mg), methyl angolensate (44 mg), xylocarpin (3.4 mg), and xylocarpin I (3.9 mg), and subfractions Ab24−Ab26 were combined and purified by preparative HPLC (MeOH−H_2O, 51:49) to afford 6-deoxy-swietenine (20.5 mg) and granaxylocarpin B (16.8 mg).

Fractions A46 to A49 were combined and purified by preparative HPLC (MeCN−H_2O, 53:47) to afford compounds **1** (45.1 mg), **2** (21.5 mg), and tigloylseneganolide A (3.3 mg).

Subfractions Ba73 to BaB81 were combined and purified by preparative HPLC (MeOH−H_2O, 76:24) to give granaxylocarpin A (1.1

Table 4. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data (δ) for Compound **8** in $CDCl_3$

position	δ_H (J in Hz)	δ_C	position	δ_H (J in Hz)	δ_C
1		85.1 qC	22	6.42 br s	109.7 CH
2		79.7 qC	23	7.39 br s	143.2 CH
3	4.58 s	83.9 CH	28	1.10 s	15.5 CH_3
4		45.4 qC	29α	1.78 d (15.0)	40.0 CH_2
5	3.31 br s	40.2 CH	29β	2.06 d (15.0)	
6	6.06 br s	71.5 CH	30	5.95 s	70.4 CH
7		169.6 qC	7-OMe	3.81 s	53.0 CH_3
8		85.6 qC	3-OH	2.70 br s	
9		86.0 qC	1,8,9-Trioxyethyl		
10		46.2 qC	1′		119.2 qC
11α	2.40 dd (14.0, 3.5)	31.6 CH_2	2′	1.66 s	21.1 CH_3
11β	1.81 br d (14.0)		2-Acetoxy		
12	4.61 br d (3.5)	69.0 CH_2	1″		169.2 qC
13		38.8 qC	2″	1.63 s	20.1 CH_3
14	2.24 br d (7.5)	43.8 CH	6-Acetoxy		
15α	2.78 dd (20.5, 10.5)	26.9 CH_2	1‴		169.7 qC
15β	3.28 br d (20.5)		2‴	2.19 s	21.4 CH_3
16		169.6 qC	12-Acetoxy		
17	5.53 s	77.1 CH	1⁗		170.9 qC
18	1.22 s	14.0 CH_3	2⁗	2.21 s	21.3 CH_3
19	1.22 s	13.7 CH_3	30-Acetoxy		
20		120.8 qC	1⁗′		169.1 qC
21	7.46 br s	140.9 CH	2⁗′	2.09 s	21.2 CH_3

Table 5. ^{1}H (500 MHz) and ^{13}C (100 MHz) NMR Data (δ) for Compounds **9** and **10** in $CDCl_3$

	9		**10**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		198.6 qC		198.6 qC
2		130.4 qC		130.4 qC
3	6.98 s	161.3 CH	6.94 s	161.1 CH
4		36.7 qC		36.7 qC
5	2.32[a]	45.4 CH	2.32[a]	45.4 CH
6	2.32[a]	34.8 CH_2	2.32[a]	34.8 CH_2
	2.49 m		2.50 m	
7		173.4 qC		173.4 qC
8		200.8 qC		200.7 qC
9		79.7 qC		79.7 qC
10	2.41 m	43.2 CH	2.41 m	43.2 qC
11α	1.79 ddd (14.6, 14.5, 4.0)	29.4 CH_2	1.78 ddd (14.5, 14.4, 4.1)	29.5 CH_2
11β	1.67 ddd (14.8, 3.6, 3.6)		1.67 ddd (14.7, 3.5, 3.5)	
12α	1.38 ddd (14.3, 3.2, 3.2)	28.4 CH_2	1.38 ddd (14.4, 3.4, 3.4)	28.5 CH_2
12β	2.29 m		2.32 m[a]	
13		41.5 qC		41.5 qC
14		156.4 qC		156.6 qC
15	6.33 s	121.6 CH	6.30 s	121.3 CH
16		163.4 qC		163.3 qC
17	5.52 s	81.4 CH	5.52 s	81.4 CH
18	1.07 s	18.6 CH_3	1.07 s	18.6 CH_3
19	1.15 d (7.0)	12.2 CH_3	1.14 d (7.0)	12.2 CH_3
20		119.3 qC		119.3 qC
21	7.61 br s	141.5 CH	7.61 br s	141.5 CH
22	6.50 br s	109.7 CH	6.50 br s	109.7 CH
23	7.44 br s	143.3 CH	7.44 br s	143.3 CH
28	1.17 s	28.1 CH_3	1.15 s	28.1 CH_3
29	1.10 s	20.6 CH_3	1.09 s	20.5 CH_3
30	6.27 s	66.3 CH	6.21 s	66.1 CH
7-OMe	3.70 s	52.0 CH_3	3.70 s	52.0 CH_3
8-OH	3.77 s		3.79 s	
30-Acyl				
1′		172.3 qC		174.8 qC
2′	2.25 m	27.5 CH_2	2.46 m	33.8 CH
3′	1.09 t (7.5)	9.1 CH_3	1.11 d (7.0)	18.8 CH_3
4′			1.09 d (7.0)	18.8 CH_3

[a] Overlapped signals assigned by HSQC and HMBC spectra without designating multiplicity.

mg), piscidinol G (4.3 mg), protoxylocarpins A (9.1 mg) and D (41.7 mg), sapelin E acetate (3.2 mg), and xylogranatins N (41.5 mg) and O (29.5 mg).

Absolute Configuration at C-2 in the 2-Methylbutyryl Group of Hainangranatumins A and B (1 and 2). A portion of hainangranatumin A (**1**, 5 mg) was dissolved in EtOH (0.5 mL) and treated with 5% KOH in H_2O (1.0 mL), with stirring at room temperature for 24 h. The reaction mixture was concentrated and partitioned between EtOAc and H_2O (3:1 v/v). After extraction with EtOAc (×3), the aqueous layer was acidified with HCl to pH 3.0 and extracted again with CH_2Cl_2 (×3). The organic layer was combined and subjected to Sephadex LH-20 CC (CH_2Cl_2−MeOH, 1:1) to provide 2-methylbutyric acid (0.7 mg), which was dried over anhydrous Na_2SO_4 and identified on the basis of its ^{1}H NMR spectrum. The absolute configuration at C-2 was suggested

as *R* by its negative α_D value {$[\alpha]^{25}_D$ −6.7 (*c* 0.03, Me_2CO)}. In the same way, the absolute configuration of C-2 in the 2-methylbutyryl group of hainangranatumin B was established as *S* by its positive α_D value {$[\alpha]^{25}_D$ +10.0 (*c* 0.04, Me_2CO)}.

X-ray Crystallographic Analysis of Hainangranatumins A and B (1 and 2). Colorless crystals of **1** and **2** were obtained in acetone. Crystal data were obtained on a Bruker Smart 1000 CCD single-crystal diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) and operating in the ω scan mode. The structure was solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Crystallographic data for **1** and **2** were deposited with the Cambridge Crystallographic Data Centre with the deposition number 779982 and 779981, respectively. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1233-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Crystal Data of 1: monoclinic, $C_{32}H_{40}O_{10}$, space group $P2_1$ with a = 8.1004(4) Å, b = 16.0763(8) Å, c = 11.7961(6) Å, V = 1514.45(13) Å^3, Z = 2, D_{calcd} = 1.282 g/cm^3, m = 0.095 mm^{-1}, and $F(000)$ = 624. Crystal size: 0.46 × 0.42 × 0.41 mm^3. Independent reflections: 3034 [R_{int} = 0.0164]. The final indices were R_1 = 0.0336, wR_2 = 0.0892 [$I > 2\sigma(I)$].

Crystal Data of 2: monoclinic, $C_{32}H_{40}O_{10}$, space group $P2_1$ with a = 7.9418(4) Å, b = 16.2706(9) Å, c = 11.6249(6) Å, V = 1491.53(14) Å^3, Z = 2, D_{calcd} = 1.302 g/cm^3, m = 0.096 mm^{-1}, and $F(000)$ = 624. Crystal size: 0.46 × 0.42 × 0.32 mm^3. Independent reflections: 3355 [R_{int} = 0.0197]. The final indices were R_1 = 0.0352, wR_2 = 0.0993 [$I > 2\sigma(I)$].

Hainangranatumin A (1): colorless crystals (acetone); mp 125−127 °C; $[\alpha]^{25}_D$ −28.6 (*c* 2.47, Me_2CO); UV (MeOH) λ_{max} 210.9 nm; for ^{1}H and ^{13}C NMR data, see Tables 1 and 2; LR-ESIMS *m/z* 607.1 $[M + Na]^+$.

Hainangranatumin B (2): colorless crystals (acetone); mp 130−131 °C; $[\alpha]^{25}_D$ −22.8 (*c* 0.88, Me_2CO); UV (MeOH) λ_{max} 210.9 nm; for ^{1}H and ^{13}C NMR data, see Tables 1 and 2; LR-ESIMS *m/z* 607.1 $[M + Na]^+$.

Hainangranatumin C (3): white, amorphous powder; $[\alpha]^{25}_D$ −11.3 (*c* 5.99, Me_2CO); UV (MeOH) λ_{max} 212.5 nm; for ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HR-TOFMS *m/z* 579.2185 [calcd for $C_{30}H_{36}O_{10}Na$ $[M + Na]^+$, 579.2206].

Hainangranatumin D (4): white, amorphous powder; $[\alpha]^{25}_D$ −37.1 (*c* 0.62, Me_2CO); UV (MeOH) λ_{max} 212.1, 236.9 nm; for ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HR-TOFMS *m/z* 567.2173 [calcd for $C_{29}H_{36}O_{10}Na$ $[M + Na]^+$, 567.2206], HR-TOFMS *m/z* 545.2371 [calcd for $C_{29}H_{37}O_{10}$ $[M + H]^+$, 545.2387].

Hainangranatumin E (5): white, amorphous powder; $[\alpha]^{25}_D$ +8.8 (*c* 0.08, Me_2CO); UV (MeCN) λ_{max} 205.1 nm; for ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HR-TOFMS *m/z* 639.2435 [calcd for $C_{32}H_{40}O_{12}Na$ $[M + Na]^+$, 639.2417].

Hainangranatumin F (6): white, amorphous powder; $[\alpha]^{25}_D$ −55.6 (*c* 0.48, Me_2CO); UV (MeCN) λ_{max} 210.9 nm; for ^{1}H and ^{13}C NMR data, see Table 3; HR-TOFMS *m/z* 581.2319 [calcd for $C_{30}H_{38}O_{10}Na$ $[M + Na]^+$, 581.2363], HR-TOFMS *m/z* 559.2549 [calcd for $C_{30}H_{39}O_{10}$ $[M + H]^+$, 559.2543].

Hainangranatumin G (7): white, amorphous powder; $[\alpha]^{25}_D$ +135.3 (*c* 0.17, Me_2CO); UV (MeCN) λ_{max} 212.5, 273.7, 315.1 nm; for ^{1}H and ^{13}C NMR data, see Table 3; HR-TOFMS *m/z* 478.2239 [calcd for $C_{28}H_{31}O_6NNa$ $[M + H]^+$, 478.2230].

Hainangranatumin H (8): white, amorphous powder; $[\alpha]^{25}_D$ −56.4 (*c* 0.80, Me_2CO); UV (MeOH) λ_{max} 209.8 nm; for ^{1}H and ^{13}C NMR data, see Table 4; HR-TOFMS *m/z* 783.2464 [calcd for $C_{37}H_{44}O_{17}Na$ $[M + Na]^+$, 783.2476], HR-TOFMS *m/z* 761.2676 [calcd for $C_{37}H_{45}O_{17}$ $[M + H]^+$, 761.2657].

Hainangranatumin I (9): white, amorphous powder; $[\alpha]^{25}_D$ +83.5 (*c* 0.31, Me_2CO); UV (MeOH) λ_{max} 240.7 nm; for ^{1}H and ^{13}C NMR data, see Table 5; HR-TOFMS *m/z* 579.2239 [calcd for $C_{30}H_{36}O_{10}Na$ $[M + Na]^+$, 579.2206].

Hainangranatumin J (10): white, amorphous powder; $[\alpha]^{25}_D$ +81.4 (*c* 1.08, Me_2CO); UV (MeOH) λ_{max} 240.7 nm; for ^{1}H and ^{13}C NMR data, see Table 5; HR-TOFMS *m/z* 593.2352 [calcd for $C_{31}H_{38}O_{10}Na$ $[M + Na]^+$, 593.2363].

Acknowledgment. Financial support for this work from the Important Project of Chinese Academy of Sciences (KSCX2-YW-R-093), the National High Technology Research and Development Program of China (863 Program) (2007AA09Z407), the National Natural Science Foundation of China (20772135), the Research Foundation for Young Talents from the South China Sea Institute of Oceanology, Chinese Academy of Sciences (M.-Y.L., SQ200802), and the Beijing Natural Science Foundation of China (7082060) is gratefully acknowledged.

Supporting Information Available: Copies of ESIMS of **1** and **2**, HR-TOFMS of **3**−**10**, ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, and NOESY spectra of compounds **1**−**10**, ^{1}H−^{1}H COSY spectra of **1**−**3**, **5**, and **8**−**10**, and X-ray crystal data for **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Wu, J.; Xiao, Q.; Xu, J.; Li, M. Y.; Pan, J. Y.; Yang, M. H. *Nat. Prod. Rep.* **2008**, *25*, 955–981.
(2) Hu, W. M.; Wu, J. *Open Nat. Prod. J.* **2010**, *3*, 1–5.
(3) Li, M. Y.; Yang, S. X.; Pan, J. Y.; Xiao, Q.; Satyanandamurty, T.; Wu, J. *J. Nat. Prod.* **2009**, *72*, 1657–1662.
(4) Li, M. Y.; Yang, X. B.; Pan, J. Y.; Feng, G.; Xiao, Q.; Sinkkonen, J.; Satyanandamurty, T.; Wu, J. *J. Nat. Prod.* **2009**, *72*, 2110–2114.
(5) Wu, J.; Yang, S. X.; Li, M. Y.; Feng, G.; Pan, J. Y.; Xiao, Q.; Sinkkonen, J.; Satyanandamurty, T. *J. Nat. Prod.* **2010**, *73*, 644–649.
(6) Cui, J.; Deng, Z.; Xu, M.; Proksch, P.; Li, Q.; Lin, W. *Helv. Chim. Acta* **2009**, *92*, 139–150.
(7) Pudhom, K.; Sommit, D.; Nuclear, P.; Ngamrojanavanich, N.; Petsom, A. *J. Nat. Prod.* **2009**, *72*, 2188–2191.
(8) Shen, L. R.; Dong, M.; Guo, D.; Yin, B. W.; Zhang, M. L.; Sha, Q. W.; Huo, C. H.; Kiyota, H.; Suzuki, N.; Cong, B. *Z. Naturforsch., C: J. Biosci.* **2009**, *64*, 37–42.
(9) Yin, B.; Huo, C.; Shen, L.; Wang, C.; Zhao, L.; Wang, Y.; Shi, Q. *Biochem. Syst. Ecol.* **2009**, *37*, 218–220.
(10) Huo, C. H.; Guo, D.; Shen, L. R.; Yin, B. W.; Sauriol, F.; Li, L. G.; Zhang, M. L.; Shi, Q. W.; Kiyota, H. *Tetrahedron Lett.* **2010**, *51*, 754–757.
(11) Zaridah, M. Z.; Idid, S. Z.; Omar, A. W.; Khozirah, S. *J. Ethnopharmacol.* **2001**, *78*, 79–84.
(12) Murata, T.; Miyase, T.; Muregi, F. W.; Naoshima-Ishibashi, Y.; Umehara, K.; Warashina, T.; Kanou, S.; Mkoji, G. M.; Terada, M.; Ishih, A. *J. Nat. Prod.* **2008**, *71*, 167–174.
(13) Adesida, G. A.; Adesogan, E. K.; Taylor, D. A. H. *Chem. Commun. (London)* **1967**, 790–791.
(14) Rao, M. M.; Krishna, E. M.; Gupta, P. S.; Singh, P. P. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **1978**, *16*, 823–825.
(15) Saewan, N.; Sutherland, J. D.; Chantrapromma, K. *Phytochemistry* **2006**, *67*, 2288–2293.
(16) Kadota, S.; Marpaung, L.; Kikuchi, T.; Ekimoto, H. *Chem. Pharm. Bull.* **1990**, *38*, 639–651.
(17) David, A. H.; Taylor, F. W. W. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1599–1602.
(18) Lee, S. M.; Olsen, J. I.; Schweizer, M. P.; Klocke, J. A. *Phytochemistry* **1988**, *27*, 2773–2775.
(19) Yin, S.; Wang, X. N.; Fan, C. Q.; Lin, L. P.; Ding, J.; Yue, J. M. *J. Nat. Prod.* **2007**, *70*, 682–685.
(20) Abdelgaleil, S. A. M.; Okamura, H.; Iwagawa, T.; Sato, A.; Miyahara, I.; Doe, M.; Nakatani, M. *Tetrahedron* **2001**, *57*, 119–126.
(21) Ng, A. S.; Fallis, A. G. *Can. J. Chem.* **1979**, *57*, 3088–3089.
(22) Balakrishna, K.; Sukumar, E.; Connolly, J. D. *Nat. Prod. Sci.* **2003**, *9*, 192–194.
(23) Naidoo, D.; Mulholland, D. A.; Randrianarivelojosia, M.; Coombes, P. H. *Biochem. Syst. Ecol.* **2003**, *31*, 1047–1050.
(24) Gan, L.-S.; Wang, X.-N.; Wu, Y.; Yue, J.-M. *J. Nat. Prod.* **2007**, *70*, 1344–1347.
(25) Okorie, D. A. H. T. *J. Chem. Soc. C* **1970**, 211–213.
(26) Cui, J. X.; Wu, J.; Deng, Z. W.; Proksch, P.; Lin, W. H. *J. Nat. Prod.* **2007**, *70*, 772–778.
(27) Wu, J.; Xiao, Q.; Huang, J. S.; Xiao, Z. H.; Qi, S. H.; Li, Q. X.; Zhang, S. *Org. Lett.* **2004**, *6*, 1841–1844.
(28) Wu, J.; Xiao, Q.; Zhang, S.; Li, X.; Xiao, Z.; Ding, H.; Li, Q. *Tetrahedron* **2005**, *61*, 8382–8389.
(29) Yin, S.; Fan, C. Q.; Wang, X. N.; Lin, L. P.; Ding, J.; Yue, J. M. *Org. Lett.* **2006**, *8*, 4935–4938.
(30) Wu, J.; Zhang, S.; Bruhn, o.; Xiao, Q.; Ding, H. *Chem.—Eur. J.* **2008**, *14*, 1129–1144.
(31) Meyers, A. I.; Knaus, G.; Kamata, K. *J. Am. Chem. Soc.* **1974**, *96*, 268–270.
(32) Brechbuhler, S.; Bu̇chi, G.; Milne, G. *J. Org. Chem.* **1967**, *32*, 2641–2642.
(33) Korver, O.; Gorkom, M. V. *Tetrahedron* **1974**, *30*, 4041–4048.
(34) Wu, J.; Zhang, S.; Xiao, Q.; Li, Q.; Huang, J.; Long, L.; Huang, L. *Tetrahedron Lett.* **2004**, *45*, 591–593.
(35) Wu, J.; Xiao, Q.; Zhang, S.; Li, X.; Xiao, Z. H.; Ding, H. X.; Li, Q. X. *Tetrahedron* **2005**, *61*, 8382–8389.

NP100395W