Granatumins A−G, Limonoids from the Seeds of a Krishna Mangrove, *Xylocarpus granatum*

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Seven new limonoids (**1**−**7**), named granatumins A−G, were isolated from seeds of an Indian mangrove (*Xylocarpus granatum*) collected from the wetlands of Krishna estuary, Andhra Pradesh. The known compounds khayasin T, tigloylseneganolide A, 6-deoxyswietenine, swietemahonolide, febrifugin A, gedunin, xylogranatinin, phaseic acid, (2*R*,3*R*)-3,4′,5,7-tetrahydroxyflavanone, and (*E*)-4-hydroxycinnamic acid were also isolated. The structures were established on the basis of spectroscopic data. Granatumins A and B are mexicanolides with *endo*-conjugated $\Delta^{8,30}$ and $\Delta^{14,15}$ double bonds, and granatumins F and G are polyhydroxylated phragmalins found previously in plants of the Meliaceae. Khayasin T exhibited moderate insecticidal activity against fifth instar larvae of *Brontispa longissima* (Gestro) at a concentration of 20 mg/L.

Limonoids, triterpene derivatives from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton, have been found only in plants of the order Rutales. They are classified by the type of four rings in the intact triterpene nucleus, and these are usually oxidized and designated as A, B, C, and D. The mangroves *Xylocarpus granatum* and *X. moluccensis* are known for producing antifeedant limonoids, especially mexicanolides and phragmalins. Previous investigations on the seeds of these two species yielded an obacunol, two phragmalins, three andirobins, and 14 mexicanolides, including xyloccensins A−K.[1−5] Previously we reported the isolation and identification of eight 8,9,30-phragmalin *ortho* esters and 13 limonoids from the bark and seeds of a Chinese mangrove, *X. granatum*.[6−8] To date, 42 mexicanolides and 23 phragmalins have been isolated from the wood, seeds, and fruits of *X. granatum* and *X. moluccensis*.[9] In the current paper we present the isolation and characterization of seven new limonoids (**1**−**7**), five mexicanolides and two phragmanlins named granatumins A−G, from seeds of the Indian mangrove *Xylocarpus granatum* König (Meliaceae), collected in the mangrove wetlands of Krishna estuary, Andhra Pradesh, India, together with 10 known compounds, khayasin T,[10] tigloylseneganolide A,[11] 6-deoxyswietenine,[12] swietemahonolide,[13] febrifugin A,[14] xylogranatinin,[15] gedunin,[16] phaseic acid,[17] (2*R*, 3*R*)-3,4′,5,7-tetrahydroxyflavanone,[18] and (*E*)-4-hydroxycinnamic acid.[19] The structures of these compounds were established on the basis of spectroscopic data or comparison with data in the literature.

Results and Discussion

Compound **1** was obtained as a white, amorphous powder. Its molecular formula of $C_{31}H_{36}O_8$ was established by a quasi-molecular ion peak in the HRTOFMS at *m*/*z* 559.2304 (calcd for $[M + Na]^+$ 559.2302), indicating that **1** had 14 degrees of unsaturation. The ^1H and ^{13}C NMR data (Tables 1 and 3) of **1** indicated that nine of the 14 elements of unsaturation came from a ketone, three ester functionalities, and five carbon−carbon double bonds. Therefore the molecule was pentacyclic. The ^{13}C NMR and DEPT experiments revealed that **1** had six methyls (one OCH_3 and five tertiary CH_3 groups), four methylenes (one olefinic), 10 methines (five olefinic), and 11 quaternary carbons (four carbonyls).

The 2D NMR studies ($^1H-^1H$ COSY, HSQC, HMBC) (Figure 1) of **1** indicated the presence of a ketone (δ_C 214.2), a methoxycarbonyl group (δ_H 3.69 s, δ_C 52.0 CH_3, 173.7), a methacryloyl group [δ_H 2.03 s, 5.75 s, 6.24 s; δ_C 18.4 CH_3, 127.1 CH_2, 135.6 qC, 166.2 qC], and a β-furanyl ring [δ_H 6.48 br s, 7.43 br s, 7.51 br s; δ_C 110.2 CH, 143.2 CH, 141.4 CH, 120.2 qC]. An α,β-unsaturated δ-lactone ring D, characterized by the following NMR data [δ_H 5.15 s, 6.18 s; δ_C 79.7 CH, 112.4 CH, 37.5 qC, 160.6 qC, 164.9 qC], was confirmed by HMBC correlations between H-15/C-13, H-15/C-14, H-15/C-16, H-17/C-13, and H-17/C-16. The above NMR studies suggested that **1** was a mexicanolide. An aliphatic methine group (δ_H 3.73 t, δ_C 48.9), having $^1H-^1H$ COSY correlations to H-3 and H-30, was attributed to CH-2. Another aliphatic methine group [δ_H 3.35 (d, $J = 9.5$ Hz), δ_C 40.2], showing a $^1H-^1H$ COSY correlation to H-6 and HMBC correlations to C-4, C-6, and C-10, was assigned to CH-5. Two protons [δ_H 2.33 br s, 2.36 (d, $J = 9.5$ Hz)], having HMBC correlations to C-5 and C-7, were attributed to H_2-6. An oxygenated methine group [δ_H 4.91 (d, $J = 9.0$ Hz), δ_C 78.5 CH], exhibiting a $^1H-^1H$ COSY cross-peak to H-2 and HMBC correlations to C-2 and C-4, was assigned to CH-3. A $\Delta^{8,30}$ double bond was suggested by HMBC correlations between H-2/C-30, H-9/C-8, H-15/C-8, H-30/C-9, and H-30/C-14. The results indicated that **1** was a mexicanolide with *endo*-conjugated $\Delta^{8,30}$ and $\Delta^{14,15}$ double bonds. The strong HMBC

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Chart 1

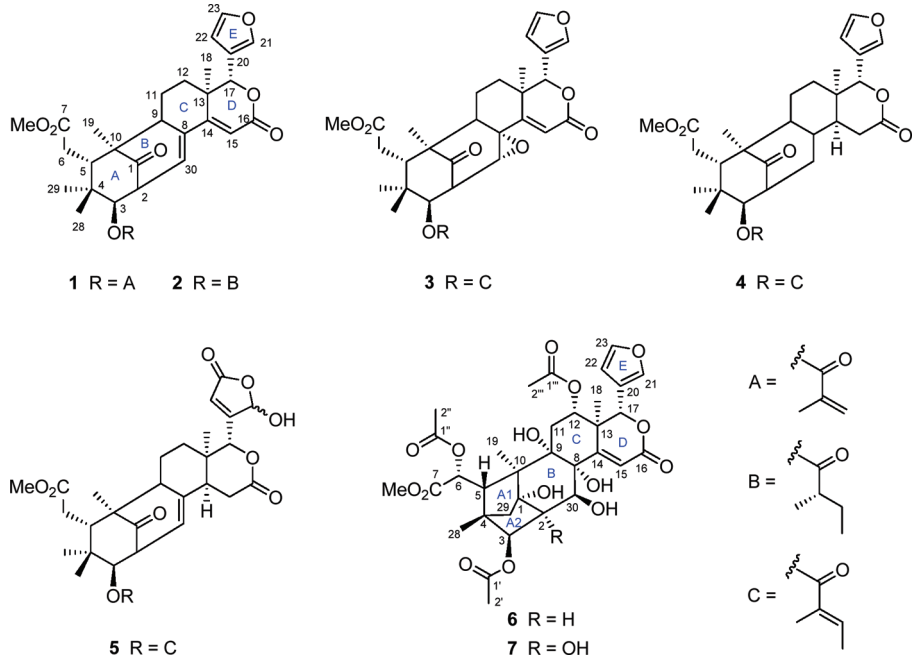

Table 1. ^{1}H NMR (500 MHz for **1**, **3**−**5** and 400 MHz for **2**) Data (δ) for Compounds **1**−**5** in $CDCl_3$ (J in Hz)

position	1	2	3	4	5
2	3.73, t (7.5)	3.65, m	3.67, dd (9.5, 2.0)	3.14, m	3.56, m
3	4.91, d (9.0)	4.90, d (9.0)	5.12, d (9.0)	4.90 d (8.5)	4.82, d (9.5)/4.85, d (9.5)
5	3.35, d (9.5)	3.30, dd (9.0, 2.0)	3.44, d (8.5)	3.77, d (9.0)	3.30, d (10.0)/3.25, d (9.5)
6a	2.33, br s	2.33, br s	2.29, br s	2.32, br s	2.37, br s
6b	2.36, d (9.5)	2.37, d (9.6)	2.34, d (9.5)	2.35, d (9.0)	2.46, d (10.0)
8				1.95, m	
9	2.29, m	2.30, m	1.99, m	1.72, m	2.23[a]
11α	1.75, m	1.74, m	1.74, m	2.30, m	2.03, dd (12.5, 4.0)
11β	1.48, m	1.49, m	1.49, m	1.58, m	1.77, m
12α	1.29, m	1.30, m	1.26, m	1.23, m	1.40, m
12β	1.94, m	1.90, m	2.02, m	1.69, m	1.67, m
14				1.89, m	2.23[a]
15α	6.18, s	6.23, s	6.12, s	2.91, dd (19.0, 7.5)	2.83, dd (18.5, 6.0)
15β				2.36, d (19.0)	2.75, br d (18.5)
17	5.15, s	5.14, s	5.22, s	5.81, s	5.43, s/5.55, s
18	1.04, s	1.05, s	1.15, s	0.97, s	1.11, s
19	1.20, s	1.19, s	1.08, s	1.09, s	1.15, s
21	7.51, br s	7.51, br s	7.51, br s	7.72, br s	6.07, br s/6.09, br s
22	6.48, br s	6.48, br s	6.50, br s	6.47, br s	6.20, br s/6.25, brs
23	7.43, br s	7.43, br s	7.45, br s	7.44, br s	
28	0.80, s	0.82, s	0.82, s	0.84, s	0.85, s
29	0.84, s	0.78, s	0.82, s	0.79, s	0.81, s/0.82, s
30α	6.26, dd (6.0, 3.0)	6.30, dd (6.0, 3.0)	3.94, d (2.0)	1.80, m	5.31, d (7.5)/5.34, br d (5.0)
30β				2.82, dd (15.0, 7.0)	
7-OMe	3.69, s	3.69, s	3.71, s	3.75, s	3.75, s/3.69, s
3-Acyl-2′		2.53, m			
3′	5.75, s	1.57, m	7.07, m	6.88, m	6.98, m
	6.24, s	1.78, m			
4′	2.03, s	1.01, t (7.5)	1.93, d (7.0)	1.77, d (7.0)	1.80, d (6.5)
5′		1.25, d (7.0)	1.95, s	1.82, s	1.82, s/1.89, s

[a] Overlapped signals assigned by ^{1}H−^{1}H COSY, HSQC, and HMBC spectra without designating multiplicity.

correlation from H-3 of the mexicanolide nucleus to the carbonyl carbon (δ_C 166.2) of the methacryloyl group disclosed its location at C-3.

The relative configuration of **1** was established on the basis of the NOESY interactions (Figure 2). The significant NOE interaction from H-3 to H_3-29, but not from H-3 to H-5, helped to establish the 3α-H and the corresponding 3β-*O*-methacryloyl group. The axial orientation of H-3 was supported by the value of its coupling constant with H-2 (J = 9 Hz). The NOE interactions between H-12α/H_3-18, H-9/H_3-18, and H-9/H_3-19 indicated their mutual *cis* relationship and the α-orientation. Similarly, NOE interactions between H-5/H-11β, H-5/H-30, and H-5/H_3-28 indicated the β-orientation of H-5, and those between H-17/H-12β suggested the β-orientation of H-17. Thus, the relative configuration of **1**, named granatumin A, was established as shown.

Compound **2** had the molecular formula $C_{32}H_{40}O_8$, as established by HRTOFMS (*m*/*z* 575.2609, calcd for $[M + Na]^+$ 575.2615). The NMR data of **2** were similar to those of **1**, but revealed the presence of a 2-methylbutyryl moiety [δ_H 1.01 (t, J = 7.5 Hz), 1.25 (d, J = 7.0 Hz), 1.57 m, 1.78 m, 2.53 m; δ_C 12.0 CH_3, 17.1 CH_3, 26.7 CH_2, 41.4 CH, 175.7 qC] (Tables 1 and 3) in place of the 3β-methacryloyl group of **1**. The 2-methylbutyryl group was corroborated by ^{1}H−^{1}H COSY correlations between H_3-4′/H_2-3′, H_2-3′/H-2′, and H-2′/H_3-5′ and HMBC interactions between H_3-

Table 2. ^{1}H NMR (400 MHz, $CDCl_3$) Data (δ) for Compounds **6** and **7** (J in Hz)

position	6	7
2	3.07, d (12.4)	
3	5.18, d (12.0)	5.04, s
5	2.61, br s	2.53, br s
6	6.43, br s	6.43, br s
11α	2.23, dd (14.0, 4.0)	2.21[a]
11β	1.89, t (14.0)	1.89, t (14.0)
12	4.89, dd (13.2, 4.0)	4.84, dd (13.5, 4.0)
15	6.23, s	6.31, s
17	5.92, s	5.95, s
18	1.64, s	1.65, s
19	1.37, s	1.41, s
21	7.44, br s	7.43, br s
22	6.55, br s	6.55, br s
23	7.38, br s	7.38, br s
28	0.95, s	0.95, s
29$_{pro\text{-}S}$	2.30, d (10.8)	2.18, d (10.5)
29$_{pro\text{-}R}$	1.44, d (10.8)	1.68, d (10.5)
30	4.42, br s	4.41, br s
7-OMe	3.74, s	3.74, s
3-OAc-2′	2.02, s	2.06, s
6-OAc-2″	2.20, s	2.21, s
12-OAc-2‴	1.55, s	1.55, s

[a] Overlapped signals assigned by ^{1}H−^{1}H COSY, HSQC, and HMBC spectra without designating multiplicity.

4′/C-3′, H_3-4′/C-2′, H_3-5′/C-2′, H_3-5′/C-1′, and H-2′/C-1′. The HMBC cross-peak from H-3 [4.90 (d, J = 9.0 Hz)] to a carbonyl carbon placed the 2-methylbutyryl group at C-3. The significant NOE interaction observed in **2**, from H-3 to H_3-29, but not from H-3 to H-5, established the 3β-orientation of the 2-methylbutyryl group. The absolute configuration at C-2′ in the 2-methylbutyryl group was determined as *S* on the basis of the positive specific rotation of the corresponding acid [[α]$^{25}_D$ +16 (*c* 0.05, Me_2CO)], obtained from alkaline hydrolysis of **2**.[20−22] Thus, granatumin B (**2**) was identified as 3-*O*-2*S*-methylbutyryl 3-demethacryloylgranatumin A.

Compound **3** had the molecular formula $C_{32}H_{38}O_9$, as established by HRTOFMS. The NMR data of **3** were similar to those of swietemahonolide,[13] except for the presence of a $\Delta^{14,15}$ double bond (δ_H 6.12 s; δ_C 118.8 CH, 160.9 qC). The existence of this double bond was supported by HMBC correlations between H-15/C-8, H-15/C-13, H-15/C-14, H-15/C-16, and H-30/C-14. Moreover, the α-orientation of the 8,30-epoxy ring in **3** was confirmed by the NOE interaction between H-30/H-15. Therefore, granatumin C (**3**) was concluded to be 14,15-dedihydroswietemahonolide.

The NMR data of **4** ($C_{32}H_{42}O_8$ by HRTOFMS) were similar to those of swietemahonolide,[13] except for the lack of the 8,30-epoxy ring [δ_H 3.22 (d, J = 2.5 Hz); δ_C 60.7, 63.4 in swietemahonolide], which was confirmed by carbon multiplicities observed in the DEPT experiment. Significant NOE interactions observed in **4** between H-9/H_3-19, H-14/H_3-18, and H-8/H-9 established their α-orientation. Thus, granatumin D (**4**) was identified as 8,30-deepoxyswietemahonolide.

Compound **5**, a white, amorphous powder, had the molecular formula $C_{32}H_{40}O_{10}$. The NMR data of **5** were similar to those of febrifugin A,[14] except for the different γ-hydroxybutenolide group substituted at C-17. The NMR data of the γ-hydroxybutenolide group in **5**, characterized by two broad proton singlets at δ_H 6.07/6.09 (H-21) and 6.20/6.25 (H-22) and by resonances at δ_C 163.2 (C-20), 97.5/99.3 (C-21), 120.5/122.0 (C-22), and 169.4 (C-23), were the same as those of kihadanin A.[23] The appearance of pairs of most proton and carbon resonances in the NMR spectra of **5** suggested the presence of C-21 epimers. Thus, the structure of granatumin E (**5**) was elucidated as shown.

Compound **6** had the molecular formula $C_{33}H_{40}O_{15}$. The NMR data of **6** were similar to those of xyloccensin Y ($C_{33}H_{42}O_{15}$),[24] except for the presence of a 12-acetyl group (δ_H 1.55 s; δ_C 19.9 CH_3, 170.6 qC), a $\Delta^{14,15}$ double bond (δ_H 6.23 s; δ_C 120.6 CH, 162.3 qC) (Tables 2 and 3), and the absence of the 30-acetyl group of xyloccensin Y (δ_H 2.19 s; δ_C 21.0 CH_3, 171.4 qC in xyloccensin Y). The HMBC correlation from H-12 (δ 4.89 dd, 13.2, 4.0) to the acetyl carbon at δ 170.6 confirmed the location of the 12-acetyl group. The existence of the $\Delta^{14,15}$ double bond was corroborated by HMBC correlations between H-15/C-8, H-15/C-14, H-15/C-16, H-17/C-14, and H_3-18/C-14 (Figure 3). Moreover, the significant NOE interactions observed in **6** from H-30 to H-2 and H-15, but not from H-30 to H-5, H-11β, and H-17, established the 30α-H and the corresponding 3β-OH group (Figure 4). Similarly, NOE interactions from H-3 to H$_{pro\text{-}R}$-29, but not from H-3 to H-5, helped to establish this 3α-H and the corresponding 3β-OAc group. Those between H-5/H-12β, H-5/H-17, and H-12β/H-17 indicated their mutual *cis* relationship and β-orientation (Figure 4). Therefore, the structure of **6**, named granatumin F, was established as shown.

Compound **7** had a molecular formula of $C_{33}H_{40}O_{16}$, established by HRTOFMS, which suggested the presence of an additional OH group when compared to the structure of **6**. Assignment of this OH group to C-2 of **7** was corroborated by the downfield chemical shift of this carbon in **7** (δ_C 76.0 qC) and HMBC correlations from H-3 and H-30 to C-2. The relative configuration of **7** was the same as that of **6**, on the basis of NOE interactions between H-5/H-12β, H-5/H-17, H-17/H-21, H-15/H_3-18, H-21/H_3-18, and H-22/H_3-18. Thus, granatumin G (**7**) was identified as 2-hydroxygranatumin F.

The obtained mexicanolides were tested for insecticidal activity using a conventional leaf disk method against the fifth instar larvae of *Brontispa longissima* (Gestro). Khayasin T, previously reported to have significant insecticidal activity against the leafcutting ants *Atta sexdens rubropilosa*,[25] exhibited moderate insecticidal activity at a concentration of 20 mg/L. Its lethal rates against the fifth instar larvae of *B. longissima* at exposure times of 48, 72, and 96 h were 17.4%, 27.8%, and 41.5%, respectively. The other compounds were less active. However, lethal rates of toosendanin at the same concentrations were 0%, 7.4%, and 7.4%, respectively.

Experimental Section

General Experimental Procedures. 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 MALDITOFMS spectra were measured on a Bruker APEX II spectrometer in positive ion mode. NMR spectra were recorded in $CDCl_3$ using Bruker AV-400 or AV-500 spectrometers with TMS as the internal standard. Preparative HPLC was carried out on ODS columns (250 × 10 mm i.d. and 250 × 4.6 mm i.d.,YMC) with a Waters 2998 photodiode array detector. For CC, silica gel (200−300 mesh) (Qingdao Mar. Chem. Ind. Co. Ltd.) and RP C_{18} gel (Cosmosil C18-PREP 140 μm, Nacalai Tesque, Kyoto, Japan) were used.

Plant Material. The seeds of *X. granatum* were collected in September 2007 at the mangrove wetlands in Krishna estuary, Andhra Pradesh, India. The identification of the plant was performed by one of the authors (T. S.). A voucher sample (No. IndianXM-02) is maintained in the Herbarium of the South China Sea Institute of Oceanology.

Extraction and Isolation. Dried seeds (10.0 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 resulting EtOAc extract (328 g) was chromatographed on silica gel and eluted using a $CHCl_3$−MeOH system (100:0−5:1) to yield 229 fractions. Fractions 63 to 70 (25.0 g) were combined and further purified by RP C_{18} CC (MeCN−H_2O, 50:50−100:0) to afford 82 subfractions. Subfractions 30 and 31 were combined and subjected to preparative HPLC (YMC-Pack ODS-5-A, 250 × 20 mm i.d. and 250 × 4.6 mm i.d., MeOH−H_2O, 50:50 to 55: 45) to yield compounds **1** (3 mg), **2**, (5 mg), **3** (3 mg), **4**, (3 mg), **5** (2 mg), **6** (3 mg), and **7** (2 mg), khayasin T[10] (4 mg), tigloylseneganolide A[11] (4 mg), 6-deoxyswietenine[12] (6 mg), swietemahonolide[13] (5 mg), febrifugin A[14] (2 mg), xylogranatinin[15] (30 mg), gedunin[16] (5 mg), phaseic acid[17] (2 mg), (2*R*,3*R*)-

Table 3. ^{13}C NMR (125 MHz for **1**, **3**−**5** and 100 MHz for **2**, **6**−**7**) Data (δ) for Compounds **1**−**7** in $CDCl_3$

position	**1** (δ, mult.)	**2** (δ, mult.)	**3** (δ, mult.)	**4** (δ, mult.)	**5** (δ, mult.)	**6** (δ, mult.)	**7** (δ, mult.)
1	214.2, qC	214.3, qC	214.2, qC	219.6, qC	216.4, qC	82.1, qC	83.8, qC
2	48.9, CH	49.1, CH	48.6, CH	48.9, CH	48.5/48.6, CH	48.3, CH	76.0, qC
3	78.5, CH	78.0, CH	77.1, CH	76.9, CH	77.0, CH	77.6, CH	86.4, CH
4	39.0, qC	38.9, qC	39.7, qC	39.8, qC	38.4/38.6, qC	46.0, qC	43.8, qC
5	40.2, CH	40.5, CH	41.8, CH	41.8, CH	42.0/42.3, CH	43.5, CH	43.6, CH
6	32.9, CH_2	33.0, CH_2	32.7, CH_2	32.8, CH_2	33.2/33.3, CH	71.6, CH	71.5, CH
7	173.7, qC	173.7, qC	173.5, qC	174.1, qC	176.4, qC	171.8, qC	171.9, qC
8	136.2, qC	136.0, qC	60.3, qC	36.6, CH	137.5/137.9, qC	70.6, qC	72.1, qC
9	54.1, CH	53.5, CH	55.9, CH	51.1, CH	56.0/56.4, CH	78.9, qC	78.0, qC
10	51.9, qC	51.8, qC	49.0, qC	50.3, qC	49.4, qC	49.9, qC	48.8, qC
11	21.8, CH_2	21.4, CH_2	21.0, CH_2	17.8, CH_2	21.1, CH_2	32.1, CH_2	32.1, CH_2
12	32.9, CH_2	32.4, CH_2	33.1, CH_2	34.5, CH_2	34.5/34.8, CH_2	68.0, CH	67.9, CH
13	37.5, qC	37.5, qC	39.1, qC	34.4, qC	36.9/37.7, qC	43.3, qC	43.3, qC
14	160.6, qC	160.6, qC	160.9, qC	41.2, CH	45.6/46.3, CH	162.3, qC	161.7, qC
15	112.4, CH	112.2, CH	118.8, CH	31.9, CH_2	29.5, CH_2	120.6, CH	120.9, CH
16	164.9, qC	164.8, qC	163.9, qC	170.2, qC	167.3, qC	164.8, qC	164.8, qC
17	79.7, CH	79.8, CH	79.3, CH	78.3, CH	75.5/75.7, CH	78.6, CH	78.6, CH
18	22.4, CH_3	22.3, CH_3	21.3, CH_3	22.6, CH_3	21.0, CH_3	15.9, CH_3	16.0, CH_3
19	15.6, CH_3	15.7, CH_3	15.8, CH_3	18.2, CH_3	16.4, CH_3	14.3, CH_3	14.5, CH_3
20	120.2, qC	120.2, qC	119.8, qC	121.2, qC	163.2, qC	121.3, qC	121.2, qC
21	141.4, CH	141.5, CH	141.7, CH	141.3, CH	97.5/99.3, CH	142.1, CH	142.1, CH
22	110.2, CH	110.2, CH	110.4, CH	109.8, CH	120.5/122.0, CH	110.4, CH	110.4, CH
23	143.2, CH	143.2, CH	143.3, CH	143.1, CH	169.4, qC	142.8, CH	142.9, CH
28	22.2, CH_3	21.8, CH_3	22.7, CH_3	22.3, CH_3	20.0, CH_3	15.7, CH_3	15.6, CH_3
29	21.0, CH_3	21.3, CH_3	22.7, CH_3	22.9, CH_3	23.2, CH_2	44.8, CH_2	41.1, CH_2
30	128.9, CH	129.0, CH	61.9, CH	25.8, CH_2	124.1/124.5, CH	66.2, CH	68.0, CH
7-OMe	52.0, CH_3	52.0, CH_3	52.1, CH_3	52.2, CH_3	53.1, CH_3	53.2, CH_3	53.3, CH_3
3-acyl-1′	166.2, qC	175.7, qC	166.6, qC	167.0, qC	166.8/167.1, qC	170.9, qC	170.3, qC
2′	135.6, qC	41.4, CH	127.9, qC	128.1, qC	127.3, qC	21.6, CH_3	21.6, CH_3
3′	127.1, CH_2	26.7, CH_2	140.0, CH	138.8, CH	140.2/140.4, CH		
4′	18.4, CH_3	12.0, CH_3	14.8, CH_3	14.6, CH_3	14.8, CH_3		
5′		17.1, CH_3	12.3, CH_3	12.0, CH_3	11.9, CH_3		
6-OAc-1″						169.1, qC	169.2, qC
2”						21.1, CH_3	21.1, CH_3
12-OAc-1‴						170.6, qC	170.7, qC
2‴						19.9, CH_3	19.9, CH_3

3,4′,5,7-tetrahydroxyflavanone[18] (3 mg), and (*E*)-4-hydroxycinnamic acid[19] (3 mg).

Granatumin A (1): white, amorphous powder; $[\alpha]^{25}_D$ +68 (*c* 0.1, Me_2CO); UV (MeCN) λ_{max} 214.5, 283.2 nm; ^1H and ^{13}C NMR data (Tables 1 and 2); HRTOFMS *m/z* 559.2304 [calcd for $C_{31}H_{36}O_8Na$ [M + Na]$^+$, 559.2302]; HRTOFMS *m/z* 537.2509 (calcd for $C_{35}H_{45}O_{11}$ [M + H]$^+$, 537.2483).

Granatumin B (2): white, amorphous powder; $[\alpha]^{25}_D$ +46 (*c* 0.56, Me_2CO); UV (MeCN) λ_{max} 210.1, 284.1 nm; ^1H and ^{13}C NMR data (see Tables 1 and 2); HRTOFMS *m/z* 575.2609 (calcd for $C_{32}H_{40}O_8Na$ [M + Na]$^+$, 575.2615).

Absolute Configuration of the 2-Methylbutyryl Group of Granatumin B (2). The hydrolysis of **2** with 6% KOH in water yielded 2-methylbutyric acid. The absolute configuration of its C-2 was suggested to be *S* by comparison of its specific rotation [$[\alpha]^{25}_D$ +16 (*c* 0.05, Me_2CO)] with those reported in the literature.[20−22]

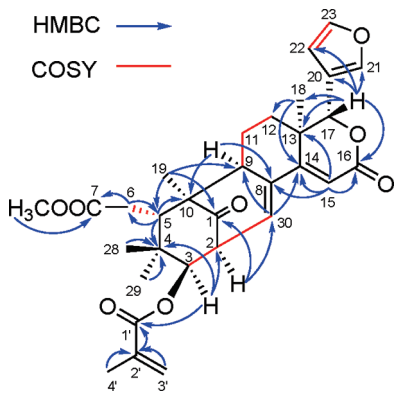

Figure 1. Selected $^1H-^1H$ COSY and HMBC correlations for granatumin A (**1**).

Granatumin C (3): white, amorphous powder; $[\alpha]^{25}_D$ +20 (*c* 0.22, Me_2CO); UV (MeCN) λ_{max} 221.6 nm; ^1H and ^{13}C NMR data (Tables 1 and 2); HRTOFMS *m/z* 589.2412 (calcd for $C_{32}H_{38}O_9Na$ [M + Na]$^+$, 589.2408); HRTOFMS *m/z* 567.2599 (calcd for $C_{36}H_{47}O_{11}$ [M + H]$^+$, 567.2589).

Granatumin D (4): white, amorphous powder; $[\alpha]^{25}_D$ −131 (*c* 0.28, Me_2CO); UV (MeCN) λ_{max} 215.7 nm; ^1H and ^{13}C NMR data (Tables 1 and 2); HRTOFMS *m/z* 577.2786 (calcd for $C_{32}H_{42}O_8Na$ [M + Na]$^+$, 577.2772).

Granatumin E (5): white, amorphous powder; $[\alpha]^{25}_D$ −101 (*c* 0.1, Me_2CO); UV (MeCN) λ_{max} 214.5 nm; ^1H and ^{13}C NMR data (Tables 1 and 2); HRTOFMS *m/z* 607.2520 (calcd for $C_{32}H_{40}O_{10}Na$ [M + Na]$^+$, 607.2514).

Granatumin F (6): white, amorphous powder; $[\alpha]^{25}_D$ +35 (*c* 0.33, Me_2CO); UV (MeCN) λ_{max} 206.2 nm; ^1H and ^{13}C NMR data (Tables 1 and 2); HRTOFMS *m/z* 699.2263 (calcd for $C_{33}H_{40}O_{15}Na$ [M + Na]$^+$, 699.2259).

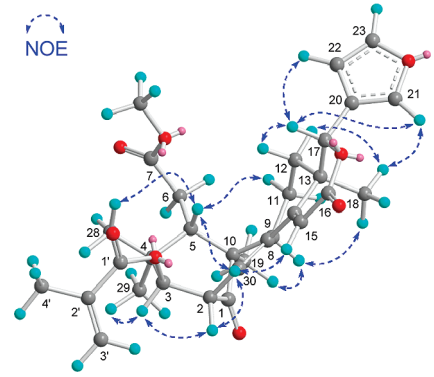

Figure 2. Diagnostic NOE correlations for granatumin A (**1**).

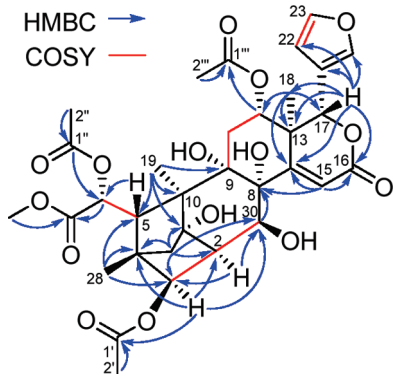

Figure 3. Selected $^{1}H-^{1}H$ COSY and HMBC correlations for granatumin F (**6**).

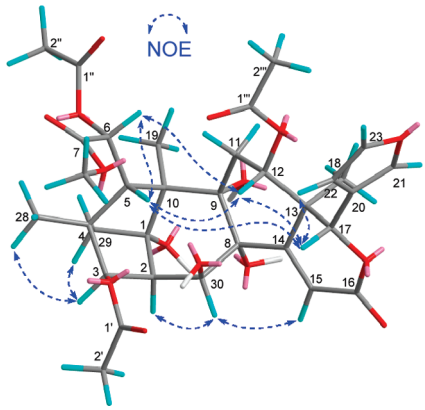

Figure 4. Diagnostic NOE correlations for granatumin F (**6**).

Granatumin G (7): white, amorphous powder; $[\alpha]^{25}_{D}$ +39 (*c* 0.12, Me_2CO); UV (MeCN) λ_{max} 212.1 nm; ^{1}H and ^{13}C NMR data (Tables 1 and 2); HRTOFMS *m/z* 715.2216 (calcd for $C_{33}H_{40}O_{16}Na$ $[M + Na]^+$, 715.2209); HRTOFMS *m/z* 731.1942 (calcd for $C_{33}H_{40}O_{16}K$ $[M + K]^+$, 731.1948).

Insecticidal Bioassay. *Brontispa longissima* (Gestro), popularly named the coconut leaf beetle, is an insect pest of coconut palms in tropical areas. It has become an increasingly serious pest of coconuts throughout various growing regions in the Pacific, especially over the last three decades. Recently it has become an insect pest on Hainan Island, China. The aim of our bioassay was to study limonoids as biopesticidal leads for the control of this pest.

The adult insects of *B. longissima* were collected from leaves of coconut trees in a coconut field in Danzhou, Hainan Island, China, where pesticides had not been applied. These adult insects were reared and propagated in the laboratory under a controlled photoperiod (12:12 h light:dark), temperature ($T = 25 \pm 1$ °C), and relative humidity (RH = 70−80%), and fed daily with coconut leaves until they reached the early stage of the fifth instar larvae, when they were used for insecticidal tests. Three groups, each containing 10 larvae, were used for the insecticidal testing of each compound. Compounds were dissolved in acetone at 20 mg mL^{-1}. Wafer discs (1 cm diameter, 1 mm thick), made from coconut leaves, were dipped in acetone solutions of each compound for three seconds and then air-dried for five minutes. After drying, discs were placed in a Petri dish with the fifth instar larvae of *B. longissima*. Acetone was chosen as the blank control. After 48, 72, or 96 h, the lethal rates of each compound against the fifth instar larvae of *B. longissima* were calculated.

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Supporting Information Available: HRTOFMS and ^{1}H and ^{13}C NMR spectra of compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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