Moluccensins A−G, Phragmalins with a Conjugated C-30 Carbonyl Group from a Krishna Mangrove, *Xylocarpus moluccensis*

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Received June 17, 2009

Seven new phragmalins with a C-30 carbonyl moiety, named moluccensins A−G (**1**−**7**), among which moluccensins A−F, possessing a $\Delta^{8,14}$ double bond, and moluccensin G (**7**), containing conjugated $\Delta^{8,9}$ and $\Delta^{14,15}$ double bonds, were isolated from the seeds of an Indian mangrove, *Xylocarpus moluccensis*. The structures of these compounds were established on the basis of single-crystal X-ray diffraction analysis and spectroscopic data. This is the first report of phragmalins with a conjugated C-30 carbonyl group.

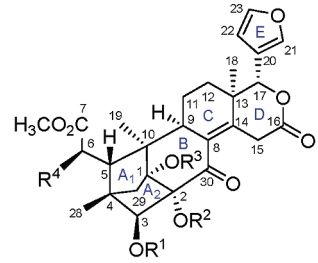
Limonoids, which have been found only in plants of the order Rutales, are triterpene derivatives from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton. They are classified by the type of four rings in the intact triterpene nucleus, and these are usually oxidized and designated as A, B, C, and D. Phragmalins, such as pseudrelones A_1 and A_2[1] isolated from *Pseudocedrela kotschyii* and khayanolides A−C[2] from *Khaya senegalensis*, have characteristic tricyclo[3.3.$1^{2,10}$.$1^{1,4}$]decane or tricyclo[4.2.$1^{10,30}$.$1^{1,4}$]decane ring systems.

The mangroves *Xylocarpus granatum* and *X. moluccensis* are known for producing antifeedant limonoids, especially phragmalins and mexicanolides. Previous investigations on the seeds of the above two Meliaceae plants uncovered an obacunol, two phragmalins, three andirobins, and 14 mexicanolides, including xyloccensins A−K.[3−7] Recently, we have reported the isolation and identification of eight unique 8,9,30-phragmalin *ortho* esters and 13 limonoids with a new carbon skeleton from the bark and seeds of the Chinese mangrove *X. granatum*, respectively.[8−10] To date, 23 phragmalins, including three 1,8,9-phragmalin *ortho* esters, eight 8,9,30-phragmalin *ortho* esters, and 12 polyhydroxylated phragmalins, were isolated from the timber, seeds, and fruits of *X. granatum* and *X. moluccensis*, together with 42 mexicanolides.[11] In the current paper, we present the isolation and characterization of seven new phragmalins with a C-30 carbonyl group, named moluccensins A−G (**1**−**7**), among which are moluccensins A−F, possessing a $\Delta^{8,14}$ double bond, and moluccensin G (**7**), containing conjugated $\Delta^{8,9}$ and $\Delta^{14,15}$ double bonds, from the seeds of the Indian mangrove *X. moluccensis*, collected in the mangrove wetlands in the Krishna estuary, Andhra Pradesh. The structures of these compounds were established on the basis of single-crystal X-ray diffraction analysis and spectroscopic data.

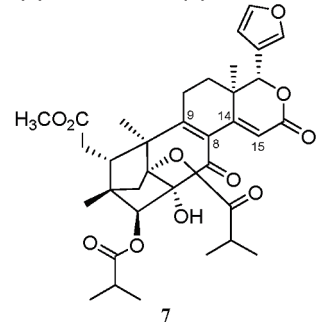
Results and Discussion

Moluccensin A (**1**), obtained as colorless crystals from acetone, had a molecular formula of $C_{35}H_{44}O_{11}$, which was established by HR-TOFMS (*m*/*z* 663.2782, calcd for $[M + Na]^+$ 663.2776). Consequently, **1** had 14 degrees of unsaturation. The ^1H and ^{13}C NMR data (Tables 1 and 2) showed that eight of the 14 elements of unsaturation came from a conjugated ketone group, three carbon−carbon double bonds, and four ester functionalities. Therefore, the molecule was hexacyclic. DEPT experiments revealed that **1** had eight methyls (a methoxy, four secondary methyls, and three tertiary methyls of the phragmalin nucleus), five methylenes, nine methines (three olefinic), and 13 quaternary carbons, including five carbonyls.

Chart 1. Structures of Compounds **1**−**7**



1: R^1 = isobutyryl R^2 = H R^3 = isobutyryl R^4 = H
2: R^1 = 2*S*-methylbutyryl R^2 = H R^3 = isobutyryl R^4 = H
3: R^1 = isobutyryl R^2 = H R^3 = 2*S*-methylbutyryl R^4 = H
4: R^1 = 2*S*-methylbutyryl R^2 = H R^3 = isobutyryl R^4 = OH
5: R^1 = isobutyryl R^2 = H R^3 = 2*S*-methylbutyryl R^4 = OH
6: R^1 = isobutyryl R^2 = isobutyryl R^3 = H R^4 = H



7

The NMR data of **1** and the information from its 2D NMR studies ($^1H-^1H$ COSY, HSQC, HMBC) (Figure 1) indicated the presence of a methoxycarbonyl (δ_H 3.63 s, δ_C 51.9 CH_3, 173.2 qC), two isobutyryl groups [δ_H 2.26 m, 1.10 (d, J = 6.8 Hz), 1.12 (d, J = 6.8 Hz), δ_C 18.8 CH_3, 19.9 CH_3, 33.9 CH, 176.0 qC; δ_H 2.52 m,

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10.1021/np9003504 CCC: $40.75
Published on Web 09/10/2009

Table 1. ^{1}H NMR (400 MHz) Data for Compounds **1**−**7** in $CDCl_3$ (*J* in Hz)

no.	1	2	3	4	5	6	7
3	5.10 s	5.13 s	5.12 s	5.06 s	5.03 s	5.28 s	5.05 s
5	2.93 d, 9.2	2.94 d, 9.2	2.95 d, 9.2	2.89, br s	2.88 br s	2.96 d, 10.0	2.84 dd, 8.8, 4.0
6a	2.35 br s	2.35 br s	2.35 br s	4.49 br s	4.49 br s	2.37 br s	2.49[a]
6b	2.35 d, 9.2	2.35 d, 9.2	2.35 d, 9.2			2.37 d, 10.0	2.41[a]
9	2.77 br s	2.77 br s	2.78 br s	2.87 br s	2.85 br s	2.63 br s	
11α	1.77 m	1.77 m	1.77 m	1.87 m	1.87 m	1.78 m	2.38[a]
11*β*	1.57 m	1.67 m	1.67 m	1.60 m	1.59 m	1.60 m	
12α	1.69 m	1.71 m	1.71 m	1.72 m	1.71 m	1.78 m	1.69, br d, 12.4
12*β*	1.32 m	1.31 m	1.33 m	1.42 m	1.42 m	1.22 m	1.48 m
15	3.87 br s	3.90 br s	3.90 br s	3.92 br s	3.88 br s	3.91 d, 14.5	7.25, s
				3.90 d, 3.6		3.84 d, 14.5	
17	5.47 s	5.47 s	5.49 s	5.40 s	5.40 s	5.43 s	5.02 s
18	1.18 s	1.19 s	1.19 s	1.18 s	1.17 s	1.19 s	1.02 s
19	1.08 s	1.09 s	1.10 s	1.37 s	1.37 s	1.14 s	1.10 s
21	7.48 br s	7.50 br s	7.49 br s	7.45 br s	7.45 br s	7.50 br s	7.50 br s
22	6.42 br s	6.44 br s	6.44 br s	6.41 br s	6.40 br s	6.44 br s	6.47 br s
23	7.42 br s	7.43, br s	7.43, br s	7.45, br s	7.45, br s	7.42, br s	7.44, br s
28	0.84 s	0.85 s	0.85 s	0.97 s	0.96 s	0.86 s	0.97 s
$29_{pro\text{-}S}$	2.24 d, 11.2	2.24 d, 11.2	2.24 d, 11.2	2.71 d, 10.8	2.71 d, 10.8	2.07 d, 11.0	2.38[a]
$29_{pro\text{-}R}$	1.98 d, 11.2	2.00 d, 11.2	2.00 d, 11.2	1.93 d, 10.8	1.93 d, 10.8	1.62 d, 11.0	2.38[a]
7-OMe	3.63 s	3.64 s	3.65 s	3.76 s	3.75 s	3.66 s	3.69 s
3-*Acyl*							
2′	2.26 m	2.05 m	2.26 m	2.07 m	2.26 m	2.30 m	2.46 m
3′	1.10 d, 6.8	1.35 m	1.09 d, 6.8	1.33 m	1.08 d, 7.2	1.12 d, 6.8	1.08 d, 7.2
		1.61 m		1.65 m			
4′	1.12 d, 6.8	0.85 t, 7.6	1.15 d, 6.8	0.88 t, 7.2	1.09 d, 7.2	1.16 d, 6.8	1.14 d, 7.2
5′		1.13 d, 6.8		1.09 d, 7.2			
Acyl	1-*Acyl*	1-*Acyl*	1-*Acyl*	1-*Acyl*	1-*Acyl*	2-*Acyl*	1-*Acyl*
2″	2.52 m	2.53 m	2.33 m	2.57 m	2.35 m	2.74 m	2.46 m
3″	1.06 d, 6.8	1.11 d, 6.8	1.41 m	1.13 d, 6.8	1.41 m	1.25 d, 7.6	1.08 d, 7.2
			1.62 m		1.62 m		
4″	1.13 d, 6.8	1.12 d, 6.8	0.89 t, 7.6	1.15 d, 6.8	0.89 t, 7.2	1.27 d, 7.6	1.14 d, 7.2
5″			1.11 d, 6.8		1.11 d, 7.2		

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

1.06 (d, $J = 6.8$ Hz), 1.13 (d, $J = 6.8$ Hz), δ_C 18.0 CH_3, 18.9 CH_3, 33.9 CH, 176.5 qC], and a *β*-furanyl ring (δ_H 6.42 br s, 7.42 br s, 7.48 br s; δ_C 109.7 CH, 120.1 qC, 141.7 CH, 143.3 CH). A *δ*-lactone ring D, characterized by NMR data [δ_H 5.47 s, 3.87 (2H, br s), δ_C 79.9 CH, 39.1 qC, 146.3 qC, 36.3 CH_2, 168.7 qC] (Tables 1 and 2), was corroborated by HMBC correlations between H_2-15/C-14, H_2-15/C-16, H-17/C-13, H-17/C-14, and H-17/C-16 (Figure 1). The above 1D and 2D NMR data strongly suggested that **1** was a phragmalin. Protons of a tertiary methyl group (δ_H 1.18 s; δ_C 19.0), showing HMBC correlations to C-12, C-13, C-14, and C-17 (Figure 1), were assigned to H_3-18. Protons of the second tertiary methyl group (δ_H 1.08 s; δ_C 17.9), exhibiting HMBC correlations to C-1, C-5, C-9, and C-10, were identified as H_3-19, and those of the third tertiary methyl group (δ_H 0.84 s; δ_C 14.5), showing HMBC correlations to C-3, C-4, and C-5 (Figure 1), were assigned to H_3-28. A pair of geminally coupled protons [δ_H 2.24 (d, 11.2 Hz) and 1.98 (d, 11.2 Hz)] of a methylene group (δ_C 41.6), exhibiting HMBC correlations to C-1, C-2, C-3, and C-4 (Figure 1), were identified as H_2-29. A hydroxy group (δ_H 4.48 br s) located at C-2 was confirmed by its strong HMBC correlation to C-2. Moreover, a $\Delta^{8,14}$ double bond was established by HMBC correlations between H_2-15/C-8, H_2-15/C-14, H-9/C-8, and H-9/C-14, and a C-30 ketone function was suggested by those between H_2-15/C-30, HO-2/C-30, H-3/C-30, and H-29/C-30 (Figure 1). The existence of the $\Delta^{8,14}$ double bond was also corroborated by the strong homoallylic coupling between H_2-15 and H-9 observed in the ^{1}H−^{1}H COSY spectrum. Furthermore, the connections of the five fragments CH-9−CH_2-11−CH_2-12, CH-5−CH_2-6, CH-22−CH-23, CH_3-3′−CH-2′−CH_3-4′, and CH_3-3″−CH-2″−CH_3-4″ were confirmed by the corresponding five homonuclear proton−proton spin systems observed in the ^{1}H−^{1}H COSY spectrum of **1** (Figure 1). The presence of the strong HMBC correlation from H-3 (δ_H 5.10, s) to C-1′ (δ_C 176.0 qC) of an isobutyryl group disclosed its location at C-3. The second isobutyryl group, however, was suggested to be attached to C-1 by its downfield chemical shift (δ_C 90.2), being lower than 90 ppm.

The relative configuration of **1** was established on the basis of the NOESY spectrum. The significant NOE interaction observed in **1** (Figure 2) from H-3 to $H_{pro\text{-}R}$-29 helped to establish this 3α-H and the corresponding 3*β*-isobutyryl group. Moreover, NOE interactions between H-5/H-11*β*, H-5/H-17, and H-17/H-21 established the *β* orientation of H-5 and H-17. Similarly, those between H-9/H_3-18, H-9/H_3-19, and H-15α/H_3-18 indicated their mutual *cis* relationship and α orientation. On the basis of the above results, the relative configuration of **1**, named moluccensin A, was established as shown in Figure 2.

The relative configuration of **1** was also confirmed by the single-crystal X-ray diffraction analysis. A computer-generated perspective drawing of the final X-ray model of **1** is given in Figure 3. The results demonstrated that **1** is a new phragmalin possessing a C-30 ketone group and a conjugated $\Delta^{8,14}$ double bond. One of the isobutyryl groups is located at C-3 in a *β* orientation and the other one at C-1 in a α orientation. Phragmalin **1** consists of six rings, designated as A_1, A_2, B, C, D, and E, among which the two five-membered rings, A_1 and A_2, adopt envelope conformations and the six-membered ring B appears in a chair conformation. However, the six-membered C ring and the *δ*-lactone D ring exhibit half-chair conformations. These conformational features instill a cage-like character to the molecule.

Moluccensin B (**2**) was isolated as a white, amorphous powder. Its molecular formula was determined to be $C_{36}H_{46}O_{11}$ by HR-TOFMS (*m/z* 677.2947, calcd for $[M + Na]^+$ 677.2932). It was larger than that of moluccensin A (**1**) by a CH_2 unit. The NMR data of **2** were similar to those of moluccensin A (**1**), except for the presence of an additional 2-methylbutyryl group [δ_H 0.85 (t, $J = 7.6$ Hz), 1.13 (d, $J = 6.8$ Hz), 1.35, m, 1.61, m, 2.05, m; δ_C 11.2 CH_3, 17.6 CH_3, 25.8 CH_2, 40.9 CH, 175.6 qC] (Tables 1 and 2) and the absence of an isobutyryl group (3*β*-isobutyryl group in

Table 2. ^{13}C NMR (100 MHz) Data for Compounds **1**−**7** in $CDCl_3$

no.	1	2	3	4	5	6	7
1	90.2 qC	90.2 qC	90.3 qC	90.0 qC	90.0 qC	88.4 qC	90.9 qC
2	79.9 qC	79.9 qC	80.0 qC	80.0 qC	79.9 qC	91.3 qC	79.1 qC
3	89.2 CH	89.2 CH	89.2 CH	90.0 CH	90.2 CH	84.0 CH	87.2 CH
4	43.4 qC	43.4 qC	43.5 qC	43.2 qC	43.1 qC	45.2 qC	45.1 qC
5	39.2 CH	39.2 CH	39.3 CH	44.7 CH	44.7 CH	37.6 CH_2	44.2 CH
6	34.0 CH_2	34.0 CH_2	34.1 CH_2	71.9 CH	71.8 CH	34.0 CH_2	33.1 CH_2
7	173.2 qC	173.2 qC	173.3 qC	175.1 qC	175.1 qC	173.6 qC	173.0 qC
8	130.7 qC	130.7 qC	130.7 qC	131.1 qC	131.1 qC	130.7 qC	122.0 qC
9	44.8 CH	44.8 CH	44.9 CH	45.9 CH	46.1 CH	40.4 CH	152.4 qC
10	47.6 qC	47.6 qC	47.7 qC	48.1 qC	48.0 qC	48.6 qC	48.5 qC
11	19.9 CH_2	19.9 CH_2	19.9 CH_2	20.1 CH_2	20.1 CH_2	18.9 CH_2	25.1 CH_2
12	30.5 CH_2	30.5 CH_2	30.6 CH_2	30.8 CH_2	30.8 CH_2	29.5 CH_2	30.2 CH_2
13	39.1 qC	39.1 qC	39.1 qC	39.2 qC	39.6 qC	38.8 qC	36.5 qC
14	146.3 qC	146.3 qC	146.4 qC	146.3 qC	146.3 qC	145.6 qC	167.1 qC
15	36.3 CH_2	36.3 CH_2	36.4 CH_2	36.3 CH_2	36.6 CH_2	35.0 CH_2	115.8 qC
16	168.7 qC	168.7 qC	168.7 qC	168.6 qC	168.6 qC	168.5 qC	165.5 qC
17	79.9 CH	79.9 CH	79.9 CH	80.2 CH	80.2 CH	79.7 CH	80.3 CH
18	19.0 CH_3	19.0 CH_3	19.1 CH_3	19.2 CH_3	19.3 CH_3	19.1 CH_3	15.7 CH_3
19	17.9 CH_3	17.9 CH_3	18.0 CH_3	19.3 CH_3	19.3 CH_3	17.8 CH_3	16.1 CH_3
20	120.1 qC	120.1 qC	120.2 qC	120.3 qC	120.2 qC	120.3 qC	120.1 qC
21	141.7 CH	141.7 CH	141.7 CH	141.1 CH	141.1 CH	141.8 CH	141.3 CH
22	109.7 CH	109.7 CH	109.7 CH	109.6 CH	109.6 CH	109.8 CH	110.1 CH
23	143.3 CH	143.3 CH	143.3 CH	143.5 CH	143.5 CH	143.1 CH	143.1 CH
28	14.5 CH_3	14.5 CH_3	14.6 CH_3	15.3 CH_3	15.2 CH_3	14.7 CH_3	16.4 CH_3
29	41.6 CH_2	41.6 CH_2	41.7 CH_2	42.8 CH_2	42.8 CH_2	41.0 CH_2	41.5 CH_2
30	198.0 qC	198.0 qC	198.1 qC	197.9 qC	198.0 qC	193.2 qC	193.1 qC
7-OMe	51.9 CH_3	51.9 CH_3	52.0 CH_3	53.2 CH_3	53.2 CH_3	52.0 CH_3	52.1 CH_3
3-*Acyl*-1′	176.0 qC	175.6 qC	176.1 qC	175.2 qC	175.7 qC	175.7 qC	175.1 qC
2′	33.9 CH	40.9 CH	33.9 CH	40.6 CH	33.8 CH	34.0 CH	34.2 CH
3′	18.8 CH_3	25.8 CH_2	18.8 CH_3	25.6 CH_2	17.9 CH_3	17.9 CH_3	18.9 CH_3
4′	19.9 CH_3	11.2 CH_3	19.9 CH_3	11.2 CH_3	19.9 CH_3	18.9 CH_3	19.0 CH_3
5′		17.6 CH_3		17.5 CH_3			
Acyl	1-*Acyl*	1-*Acyl*	1-*Acyl*	1-*Acyl*	1-*Acyl*	2-*Acyl*	1-*Acyl*
1″	176.5 qC	176.5 qC	176.2 qC	176.8 qC	176.5 qC	179.4 qC	175.6 qC
2″	33.9 CH	34.0 CH	41.0 CH	34.0 CH	41.0 CH	34.0 CH	34.2 CH
3″	18.0 CH_3	18.9 CH_3	26.6 CH_2	18.9 CH_3	26.6 CH_2	18.1 CH_3	18.9 CH_3
4″	18.9 CH_3	19.0 CH_3	11.6 CH_3	19.0 CH_3	11.6 CH_3	19.8 CH_3	19.1 CH_3
5″			16.7 CH_3		16.8 CH_3		

1). The existence of the above 2-methylbutyryl group was corroborated by $^1H-^1H$ 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_3-4′/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 (δ_H 5.13, s) of **2** to the carbonyl carbon of this 2-methylbutyryl group suggested that this acyl function was attached to C-3. Moreover, the significant NOE interaction from H-3 to $H_{pro\text{-}R}$-29 helped to establish this 3α-H and the corresponding 3β-2-methylbutyryl group. On the basis of the result that the literature α_D value of (*R*)-2-methylbutyric acid is negative (−14.3)[12] and that of (*S*)-2-methylbutyric acid is positive (+19.3, 18.9),[13,14] the absolute configuration at C-2 in the 2-methylbutyric group of **2** could be determined according to the specific rotation of its acid, which was obtained as a 1:1 mixture with isobutanoic acid from the alkaline hydrolysis of **2**. Since isobutanoic acid is optically inactive, the absolute configuration at C-2 in methylbutyric acid was suggested as *S* by the α_D value [+10 (*c* 0.16, Me_2CO)] of this mixture. Thus, moluccensin B (**2**) was identified as 3-*O*-2*S*-methylbutyryl-3-deisobutyryloxymoluccensin A.

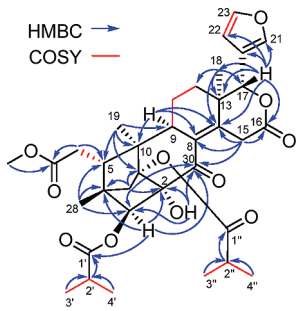

Figure 1. Selected COSY and HMBC correlations for moluccensin A (**1**).

Moluccensin C (**3**), a white, amorphous powder, had a molecular formula of $C_{36}H_{46}O_{11}$, established by HR-TOFMS (*m*/*z* 677.2966,

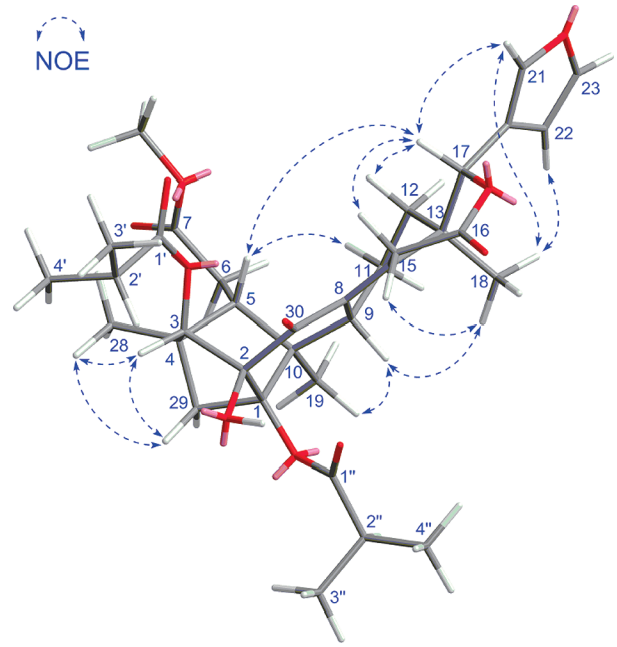

Figure 2. Diagnostic NOE correlations for moluccensin A (**1**).

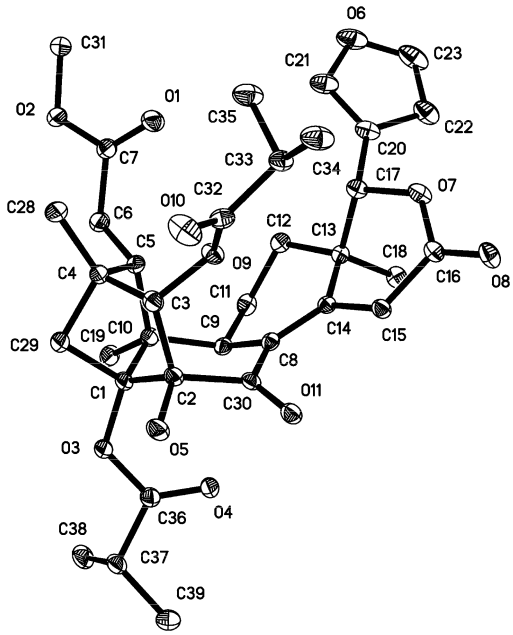

Figure 3. Perspective drawing of the X-ray structure of moluccensin A (**1**).

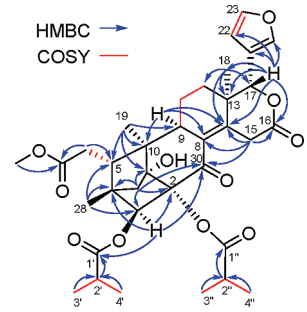

Figure 4. Selected COSY and HMBC correlations for moluccensin F (**6**).

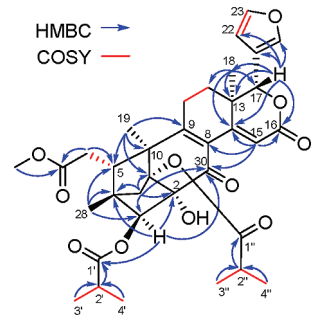

Figure 5. Selected COSY and HMBC correlations for moluccensin G (**7**).

calcd for $[M + Na]^+$ 677.2932), being the same as that of moluccensin B (**2**). The NMR data of **3** were similar to those of moluccensin A (**1**), except for the presence of one more 2-methylbutyryl group [δ_H 0.89 (t, J = 7.6 Hz), 1.11 (d, J = 6.8 Hz), 1.41, m, 1.62, m, 2.33, m; δ_C 7.6 CH_3, 16.7 CH_3, 26.6 CH_2, 41.0 CH, 176.2 qC] and the absence of an isobutyryl group (1-isobutyryl group in **1**). A 3β-isobutyryl group of **3**, being the same as that in **1**, was confirmed by the corresponding $^1H-^1H$ COSY, HMBC, and NOE correlations to those in **1**. Consequently, the 2-methylbutyryl group was located at C-1. The absolute configuration at C-2 of this group was characterized as *S* by the same method applied to **2**. On the basis of the above results, moluccensin C was concluded to be 1-*O*-2*S*-methylbutyryl-1-deisobutyryloxymoluccensin A.

Moluccensin D (**4**) was isolated as a white, amorphous powder. Its molecular formula was determined as $C_{36}H_{46}O_{12}$ by HR-TOFMS (*m*/*z* 693.2902, calcd for $[M + Na]^+$ 693.2882). It differed from that of moluccensin B (**2**) by an additional oxygen atom. The NMR data of **4** were similar to those of moluccensin B (**2**), except for the presence of one more oxygenated methine group (δ_H 4.49 br s, δ_C 71.9) (Tables 1 and 2) and the absence of a methylene group [CH_2-6 in **2**; δ_H 2.35 br s, 2.35 (d, 9.2); δ_C 34.0]. It was suggested that a hydroxy group was attached to C-6. The existence of this hydroxy group was further corroborated by the $^1H-^1H$ COSY correlation between H-5/H-6 and HMBC interactions between H-5/C-6, H-6/C-5, and H-6/C-7. Moreover, the α orientation of H-6 was determined from its NOE correlations to H_3-28 and H_3-19. This result was in agreement with two broad singlets for H-5 and H-6 in the ^1H NMR spectrum of **4**, and these were the same as those in xyloccensin O.[8] Therefore, the structure of moluccensin D (**4**) was identified as 6β-hydroxymoluccensin B.

Moluccensin E (**5**), a white, amorphous powder, had a molecular formula of $C_{36}H_{46}O_{12}$, the same as that of moluccensin D (**4**). The NMR data of **5** were similar to those of moluccensin C (**3**), except for the presence of one more oxygenated methine group (δ_H 4.49 br s, δ_C 71.8) and the absence of a methylene function [CH_2-6 in **3**; δ_H 2.35 br s, 2.35 (d, 9.2); δ_C 34.1] (Tables 1 and 2). This suggested that a hydroxy group was attached to C-6. The existence of this hydroxy group and its β orientation were further confirmed by $^1H-^1H$ COSY, HMBC, and NOE interactions similar to those in moluccensin D (**4**). On the basis of the above results, the structure of moluccensin E (**5**) was elucidated as 6β-hydroxymoluccensin C.

Moluccensin F (**6**) was isolated as a white, amorphous powder. Its molecular formula was established as $C_{35}H_{44}O_{11}$, the same as that of moluccensin A. Two isobutyryl groups were present, and one [δ_H 2.30 m, 1.12 (d, J = 6.8 Hz), 1.16 (d, J = 6.8 Hz), δ_C 17.9 CH_3, 18.9 CH_3, 34.0 CH, 175.7 qC] (Tables 1 and 2) was attached to C-3 in a β orientation deduced by $^1H-^1H$ COSY, HMBC (Figure 4), and NOE interactions as for moluccensin A. The second isobutyryl group, however, was suggested to be connected at C-2 by the downfield chemical shift of C-2 (δ_C 91.3) and upfield chemical shift of C-1 (δ_C 88.4). The relative configuration of **6** was established as the same as that of moluccensin A on the basis of the NOE correlations. Therefore, the structure of moluccensin F (**6**) was identified as 2-*O*-isobutyryl-1-deisobutyryl-moluccensin A.

Moluccensin G (**7**), a white, amorphous powder, had a molecular formula of $C_{35}H_{42}O_{11}$, established by HR-TOFMS (*m*/*z* 661.2635, calcd for $[M + Na]^+$ 661.2619), i.e., two mass units (−2H) less than that of moluccensin A. Two conjugated double bonds, $\Delta^{8,9}$ and $\Delta^{14,15}$, were present in **7**, together with a C-30 carbonyl group. These findings were corroborated by the downfield-shifted H-15 (δ_H 7.25 s) and its HMBC correlations to C-8, C-14, and C-30. Further evidence could be found from HMBC correlations between H_3-19/C-9, H-3/C-30, and H_2-29/C-30. Two isobutyryl groups, among which one was located at C-3 in a β orientation and the other one at C-1 in a α orientation, were confirmed by $^1H-^1H$ COSY correlations between H-3′/H-2′, H-2′/H-4′, H-3″/H-2″, and H-2″/H-4″, NOE interactions between H-3/$H_{pro\text{-}R}$-29 and H-3/H_3-28, the HMBC cross-peak between H-3/H-1′ (Figures 5 and 6), and the downfield chemical shift of C-1 (δ_C 90.9) in **7**, being similar to those in moluccensin A. Moreover, the relative configuration of **7** was established to be the same as moluccensin A on the basis of NOE correlations between H-5/H-11β, H-5/H-17, H-17/H-21, H-15/H_3-18, H-21/H_3-18, and H-22/H_3-18 (Figure 6). Thus, the structure of moluccensin G (**7**) was identified as $\Delta^{8,9}$, $\Delta^{14,15}$-moluccensin A.

This is the first report of phragmalins possessing a C-30 carbonyl group in conjugation with a $\Delta^{8,14}$ double bond or $\Delta^{8,9}$, $\Delta^{14,15}$ double bonds. This study demonstrated that *X. moluccensis* is a new source for the production of new limonoids, especially phragmalins.

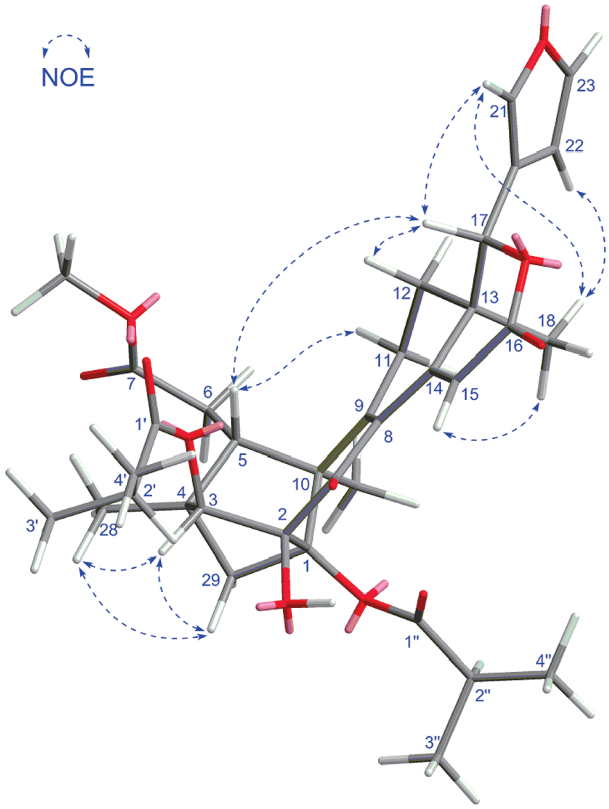

Figure 6. Diagnostic NOE correlations for moluccensin G (**7**).

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-400 spectrometer with TMS as the internal standard. MALDITOFMS spectra were measured on a Bruker APEX II spectrometer in positive ion mode. 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 Marine Chemical 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. moluccensis* were collected in October 2007 at the mangrove wetlands in Krishna estuary, Andhra Pradesh, India. The identification of the plant was performed by Mr. Tirumani Satyanandamurty, principal at Government Degree College at Amadala Valasa, Srikakulam Dist., Andhra Pradesh, India. A voucher sample (No. IndianXM-01) is maintained in the Herbarium of the South China Sea Institute of Oceanology.

Extraction and Isolation. The dried seeds (7.0 kg) of *X. moluccensis* 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 (320 g) was chromatographed on Si gel CC and eluted using a $CHCl_3$−MeOH system (100:0−5:1) to yield 230 fractions. Fractions 70 to 80 (19.0 g) were combined and further purified with RP C_{18} CC (MeCN−H_2O, 50:50−100:0) to afford 60 subfractions. Then subfractions 27 to 37 were combined and further purified with 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 moluccensins A (**1**, 21.1 mg), B (**2**, 35.6 mg), C (**3**, 14.0 mg), D (**4**, 5.0 mg), E (**5**, 22.2 mg), F (**6**, 19.3 mg), and G (**7**, 7.2 mg).

Absolute Configuration of C-2 in the 2-Methylbutyryl Group of Moluccensins B−E (2−5). A portion of moluccensin B (**2**, 7 mg) was dissolved in EtOH (0.5 mL) and treated with 6% KOH in H_2O (1 mL) with stirring at room temperature for 24 h. The reaction mixture was concentrated and partitioned between EtOAc and H_2O (3:1). After extracting with EtOAc (×3), the aqueous layer was acidified with HCl to pH 3.0 and extracted with CH_2Cl_2 (×3). The organic layer was combined, purified by Sephadex LH-20 CC (CH_2Cl_2−MeOH, 1:1), and dried over anhydrous Na_2SO_4 to provide a mixture of 1:1 isobutanoic acid and 2-methylbutyric acid (1.6 mg), which were identified on the basis of their MS. Since isobutanoic acid is optically inactive, the absolute configuration at C-2 in 2-methylbutyric acid was suggested as *S* by the α_D value [+10 (*c* 0.16, Me_2CO)] of the above mixture. In the same way, the absolute configuration of C-2 in the 2-methylbutyryl group of moluccensins C−E (**3**−**5**) was also proved as *S*.

X-ray Crystallographic Analysis of Moluccensin A (1). A colorless crystal of **1** was obtained in Me_2CO. Crystal data were obtained on a Bruker Smart 1000 CCD system 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** have been deposited with the Cambridge Crystallographic Data Centre with the deposition number CCDC 743509. 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: orthorhombic, $C_{35}H_{44}O_{11}$, space group $P2_12_12_1$ with a = 8.8804(4) Å, b = 16.4376(8) Å, c = 22.1252(10) Å, V = 3229.7(3) Å^3, Z = 4, D_{calcd} = 1.318 g/cm^3, m = 0.098 mm^{-1}, and $F(000)$ = 1368. Crystal size: 0.48 × 0.38 × 0.37 mm^3. Independent reflections: 3964 with R_{int} = 0.0284. Observed reflections: 3964 with [$I > 4\sigma(I)$]. The final indices were R_1 = 0.0374 and wR_2 = 0.0918 [$I > 2\sigma(I)$].

Moluccensin A (1): colorless crystals (Me_2CO); mp 88−90 °C; $[\alpha]^{25}_D$ +1 (*c* 2.11, Me_2CO); UV (MeCN) λ_{max} 208.6, 255.9 nm; ^{1}H and ^{13}C NMR data (see Tables 1, 2); HR-TOFMS *m/z* 663.2782 [calcd for $C_{35}H_{44}O_{11}Na$ [M + Na]$^+$, 663.2776], HR-TOFMS *m/z* 641.2966 [calcd for $C_{35}H_{45}O_{11}$ [M + H]$^+$, 641.2962].

Moluccensin B (2): white, amorphous powder; $[\alpha]^{25}_D$ −1 (*c* 3.56, Me_2CO); UV (MeCN) λ_{max} 208.5, 254.7 nm; ^{1}H and ^{13}C NMR data (see Tables 1, 2); HR-TOFMS *m/z* 677.2947 [calcd for $C_{36}H_{46}O_{11}Na$ [M + Na]$^+$, 677.2932], HR-TOFMS *m/z* 655.3153 [calcd for $C_{36}H_{47}O_{11}$ [M + H]$^+$, 655.3113].

Moluccensin C (3): white, amorphous powder; $[\alpha]^{25}_D$ +4 (*c* 1.4, Me_2CO); UV (MeCN) λ_{max} 208.5, 254.7 nm; ^{1}H and ^{13}C NMR data (see Tables 1, 2); HR-TOFMS *m/z* 677.2966 [calcd for $C_{36}H_{46}O_{11}Na$ [M + Na]$^+$, 677.2932], HR-TOFMS *m/z* 655.3131 [calcd for $C_{36}H_{47}O_{11}$ [M + H]$^+$, 655.3113].

Moluccensin D (4): white, amorphous powder; $[\alpha]^{25}_D$ −3 (*c* 0.1, Me_2CO); UV (MeCN) λ_{max} 208.5, 254.7 nm; ^{1}H and ^{13}C NMR data (see Tables 1, 2); HR-TOFMS *m/z* 693.2902 [calcd for $C_{36}H_{46}O_{12}Na$ [M + Na]$^+$, 693.2882], HR-TOFMS *m/z* 671.3075 [calcd for $C_{36}H_{47}O_{12}$ [M + H]$^+$, 671.3062].

Moluccensin E (5): white, amorphous powder; $[\alpha]^{25}_D$ −1 (*c* 2.22, Me_2CO); UV (MeCN) λ_{max} 208.6, 255.9 nm; ^{1}H and ^{13}C NMR data (see Tables 1, 2); HR-TOFMS *m/z* 671.3082 [calcd for $C_{36}H_{47}O_{12}$ [M + H]$^+$, 671.3062], HR-TOFMS *m/z* 688.3361 [calcd for $C_{36}H_{50}O_{12}N$ [M + NH_4]$^+$, 688.3328].

Moluccensin F (6): white, amorphous powder; $[\alpha]^{25}_D$ +1 (*c* 1.93, Me_2CO); UV (MeCN) λ_{max} 208.6, 255.9 nm; ^{1}H and ^{13}C NMR data (see Tables 1, 2); HR-TOFMS *m/z* 663.2773 [calcd for $C_{35}H_{44}O_{11}Na$ [M + Na]$^+$, 663.2776], HR-TOFMS *m/z* 641.2988 [calcd for $C_{35}H_{45}O_{11}$ [M + H]$^+$, 641.2956].

Moluccensin G (7): white, amorphous powder; $[\alpha]^{25}_D$ + 16 (*c* 0.72, Me_2CO); UV (MeCN) λ_{max} 208.6, 285.6 nm; ^{1}H and ^{13}C NMR data (see Tables 1, 2); HR-TOFMS *m/z* 661.2635 [calcd for $C_{35}H_{42}O_{11}Na$ [M + Na]$^+$, 661.2619], HR-TOFMS *m/z* 639.2828 [calcd for $C_{35}H_{43}O_{11}$ [M + H]$^+$, 639.2805].

Acknowledgment. Financial support for this work from the Important Project of Chinese Academy of Sciences (KSCX2-YW-R-093, KZCX2-YW-216), the National High Technology Research and Development Program of China (863 Program) (2007AA09Z407), the National Natural Science Foundation of China (20772135), and the Research Foundation for Young Talents from the South China Sea Institute of Oceanology, Chinese Academy of Sciences (Min-Yi Li, SQ200802), is gratefully acknowledged. We are grateful to Mr. T.

Satyanandamurty and Mr. T. Srikanth (Acharya Nagarjuna University, Guntur, Andhra Pradesh, India) for their help and assistance in the collection and identification of the seeds of *X. moluccensis*. We are also thankful to Mr. M. Srinivas for his assistance in the fieldwork in collection of the seeds of *X. moluccensis*.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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NP9003504