

New Limonoids from the Seeds of *Xylocarpus granatum*

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ABSTRACT

Three novel limonoids, 2,3-dideacetylxylococcin S (**1**), 30-deacetylxylococcin W (**2**) and 7-hydroxy-3-oxo-21 β -methoxy-24,25,26,27-tetranortirucall-1,14-dien-23(21)-lactone (**3**), were isolated from the seeds of the Chinese mangrove, *Xylocarpus granatum*. The structures were elucidated on the basis of one- and two-dimensional NMR (including ¹H- ¹³C-NMR, DEPT, ¹H, ¹H-COSY, HSQC, HMBC, and NOESY) and confirmed by high-resolution mass spectrometry.

Keywords: NMR; ¹H; ¹³C; *Xylocarpus granatum*; limonoid; chemical constituents;

Introduction

Xylocarpus granatum Koenig, a marine mangrove plant distributed mainly along the

seashore along the Indian Ocean and in Southeast Asia, is used as a folk medicine in Southeast Asia for the treatment of diarrhea, cholera, and fever diseases such as malaria and also as an antifeedant [1]. Since the first limonoid, gedunin, was reported from this plant [2], the unique structural patterns of limonoids have attracted considerable attention from medicinal chemists as well as chemical biologists because of their fascinating structural diversity and important biological activities. As a result, more than 50 limonoid derivatives have been isolated from *X. granatum*, and they have been classified into phragmalin-, mexicanolide-, obacunol-, and andirobin-types [3-8].

Previously investigation by our group have resulted in the isolation and identification of 3 new limonoids from the seeds of a Chinese mangrove *Xylocarpus granatum* [9,10]. Further investigation on the fruit of the same plant resulted in the discovery of three novel compounds, 2,3-dideacetylxyloccensin **S** (**1**), 30-deacetylxyloccensin **W** (**2**) and 7-hydroxy-3-oxo-21 β -methoxy-24,25,26,27-tetranortirucall-1,14-dien-23(21)-lactone (**3**) (*Fig. 1*). Herein, details of the isolation and structure elucidation of these three novel compounds are presented.

Results and discussion

2,3-dideacetylxyloccensin **S** (**1**) was obtained as a white power. The molecular formula was deduced as C₃₁H₃₆O₁₄ with 14 degrees of unsaturation by HR-TOF-MS *m/z*: ((M⁺) *m/z* 632.2109, calc. 632.2105). The ¹³C NMR spectrum revealed that **1** contains six olefinic C-atoms and three CO groups. Therefore, the remaining eight unsaturations demonstrated that **1** consisting of eight rings. The ¹H-NMR, ¹³C-NMR spectra (*Table 1*) showed the presence of six Me groups, two CH₂ groups, ten CH groups (five O-bearing and four olefinic ones), and 13 quaternary C-atoms (four O-bearing, three esters and two olefinic C-atoms). In addition, four OH groups (δ (H) 2.58 (br. *s*); δ (H) 3.40 (*s*); δ (H) 3.48 (br. *s*); δ (H) 3.53 (*s*)), three tertiary Me groups (δ (H) 1.57 (*s*), 1.53 (*s*), and 1.00 (*s*); δ (C) 14.0, 16.8, and 15.1), one MeO group (δ (H) 3.84; δ (C) 52.6), and a β -substituted furyl ring (δ (H) 6.53 (br. *s*), 7.40 (*s*), and 7.40 (*s*); δ (C) 110.0, 141.4, 142.8, and 121.2) were distinguished by the ¹H- and ¹³C-NMR data. The afore mentioned spectroscopic data implied **1** was a type of phragmalin, consisting of eight rings, designated as *A*₁, *A*₂, *B*, *C*, *D*, *E*, *F* and *G*. The structural was determined by analysis of the ¹H, ¹H-COSY, HSQC, and HMBC data of **1**. It was elucidated by analysis of the spectroscopic data starting from ring *A*₁ and *A*₂, the HMBCs

between H-C(3)/C(4), Me(29)/C(3), Me(29)/C(4), Me(29)/C(5), Me(29)/C(28), Me(19)/C(5), Me(19)/C(1) and Me(19)/C(10) indicated A_1 and A_2 ring was like showing in *Fig. 2*. The HMBC cross-peaks from H-C(17) to C(21), C(22), and C(22), from Me(18) to C(13) and C(17), from H-C(15) to C(8) and C(13), indicated the situation of C , D , and E rings. The relative configuration of **1** was defined on the basis of the NOESY spectrum and the three-dimensional drawing generated by MM2 calculation was shown in *Fig. 3*. The H-C(17) had NOE with H-C(12), but had no NOE with Me(18), Me(18) had NOE with H-C(22) indicated that β -furan ring, Me(18) and 12-OH on the same side. H-C(30) had NOE with H-C(15) suggested that ring D exhibited a half-chair conformation. The H-C(6) had NOE with Me(19) and Me(28) had NOE with Me(29) indicated Me(19) and 6-OH on the opposite side and the two five-carbocyclic rings (A_1 and A_2) adopted the envelope conformations. Based on the above results, the relative stereochemistry of **1** was elucidated as shown in *Fig. 3*. Xylocensin S, 2,3-diacetyl of **1**, was isolated from the this plant in 2005[11].

30-deacetylxylocensin W (**2**) was obtained as a white power. The molecular formula was deduced as $C_{27}H_{34}O_9$ with 11 degrees of unsaturation by HR-TOF-MS m/z : [(M^+) m/z 502.2208, calc. 502.2203]. The ^{13}C -NMR spectrum revealed that **2** contains four olefinic C-atoms and three CO groups. Therefore, the remaining six unsaturations demonstrated that **2** consisting of six rings. The 1H -NMR, ^{13}C -NMR spectra (*Table 2*) showed the presence of five Me groups, four CH_2 groups, 9 CH groups (3 O-bearing and three olefinic ones), and 9 quaternary C-atoms (two O-bearing, two esters and one olefinic C-atoms). In addition, two OH groups ($\delta(H)$ 1.67 (*s*); $\delta(H)$ 3.03 (*br. s*)], four tertiary Me groups ($\delta(H)$ 0.99 (*s*), 0.98 (*s*), 1.09 (*s*), and 0.67 (*s*); $\delta(C)$ 15.3, 16.2, 27.1 and 19.8), one MeO group ($\delta(H)$ 3.71; $\delta(C)$ 51.7), and a β -substituted furyl ring ($\delta(H)$ 6.51 (*br. dd*), 7.46 (*s*), and 7.58 (*br. s*); $\delta(C)$ 109.8, 142.7, 140.7, and 120.4] were distinguished by the 1H - and ^{13}C -NMR data. The structural was determined by analysis of the 1H , 1H -COSY, HSQC, and HMBC data of **2**. It was elucidated by analysis of the spectroscopic data starting from ring A , the HMBCs between Me(28)/C(3), Me(28)/C(4), Me(28)/C(5), Me(29)/C(3), Me(29)/C(4), Me(29)/C(5), Me(19)/C(5) and Me(19)/C(1) indicated A ring was like showing in *Fig. 4*. The HMBC cross-peaks from H-C(17) to C(21), C(22), and C(22), from Me(18) to C(13) and C(17), from H-C(15a) to C(13), C(14) and C(16), from H-C(15b) to C(14) and C(8), indicated the

situation of *C*, *D* and *E* rings. The relative configuration of **2** was defined on the basis of the NOESY spectrum and the three-dimensional drawing generated by MM2 calculation was shown in *Fig. 5*. The H-C(17) had NOE with H-C(11a), but had no NOE with Me(18), Me(18) had NOE with Me(22) indicated that β -furan ring, Me(18) on the same side. The Me(19) had NOE with H-C(9), Me(19) had no NOE with H-C(5) suggested that Me(19) and H-C(9) on the same side, Me(19) and H-C(5) on the opposite side. Based on the above results, the relative stereochemistry of **2** was elucidated as shown in *Fig. 5*. Xylococcin W, 30-acetyl of **2**, was isolated from the this plant in 2006[12].

7-hydroxy-3-oxo-21 β -methoxy-24,25,26,27-tetranortirucall-1,14-dien-23(21)-lactone (**3**) was obtained as a white powder. The molecular formula was deduced as C₂₇H₃₈O₅ with 9 degrees of unsaturation by HR-TOF-MS *m/z*: [(M⁺) *m/z* 442.2717, calc. 442.2719]. The ¹³C-NMR spectrum revealed that **3** contains four olefinic C-atoms and two CO groups. Therefore, the remaining five unsaturations demonstrated that **3** consisting of five rings. The ¹H-NMR, ¹³C-NMR spectra (*Table 3*) showed the presence of six Me groups, five CH₂ groups, nine CH groups (two O-bearing and three olefinic ones), and 7 quaternary C-atoms (one esters and one olefinic C-atoms). In addition, five tertiary Me groups [δ (H) 1.04 (s), 1.18 (s), 1.14 (s), 1.18(s), and 1.11 (s); δ (C) 27.3, 19.9, 18.6, 26.8, and 21.2], one MeO group (δ (H) 3.39; δ (C) 54.8), and a five-membered lactone (δ (H) 2.21, 2.42 (*m*), and 4.79 (*d*); δ (C) 33.8, 43.9, and 175.4) were distinguished by the ¹H- and ¹³C-NMR data. The HMBCs between Me(18)/C(12), Me(18)/C(14), Me(18)/C(17), Me(19)/C(1), Me(19)/C(5), Me(19)/C(9), H-C(30)/C(7), H-C(30)/C(9), H-C(30)/C-14, Me(28)/C(3), Me(28)/C(5) and Me(28)/C(29) indicated **3** was a typical tetracyclic tetranortriterpenoid with a five-membered lactone at C(17). (*Fig. 6*) The relative configuration of **3** was defined on the basis of the NOESY spectrum and the three-dimensional drawing generated by MM2 calculation was shown in *Fig. 7*. The Me(28) had NOE with H-C(5), H-C(5) had NOE with Me(18), and Me(18) had NOE with H-C(20) indicated that Me(28), H-C(5) and Me(18) on the same side. The Me(19) had NOE with H-C(30), Me(29), and H-C(11) suggested that the relative stereochemistry of **3** was elucidated as shown in *Fig. 7*.

Experimental

General. Chromatography: Silica gel (SiO₂, 200-300 mesh; *Qingdao Marine Chemical*

Factory, P. R. China). Semiprep HPLC: *Waters Delta Prep 3000* pump, *UV 2487* detector, and *Whatman partisil 10 ODS-2* (9.4 × 250 mm) column. Optical rotation: *Jasco DIP-370*. NMR: *Bruker AV-600*; at 600.17 MHz (¹H) and 150.93 MHz (¹³C) in CDCl₃, δ in ppm rel. to Me₄Si as an internal standard, *J* in Hz. MS: *Bruker APEX II* spectrometer. MS: *Applied Biosystems QStar XL QqTOF (ESI)*.

Plant Material. Seeds of *X. granatum* were collected in March 2006 at Hainan Island, Southern China, dried at ambient tempe., and identified by Dr. *Wen-Qing Wang*, School of Life Sciences, Xia-men University, P. R. China. Several voucher specimen (No. HEBNMC-2006-1) has been deposited in the herbarium of School of Pharmaceutical Sciences, Hebei Medical University, P. R. China.

Extraction and Isolation. Dried seeds (5 kg) of *X. granatum* were extracted with 95% EtOH at room temperature. After evaporation of the solvent under reduced pressure, the residue was suspended in H₂O and extracted with petroleum ether (PE) and CH₂Cl₂, successively. The CH₂Cl₂ extract (120 g) was chromatographed on SiO₂ and eluted using a PE/AcOEt system (30:1 to 1:10) to yield nine fractions. *Fr. 5* (10 g) was subjected to SiO₂ CC by using PE/acetone (3:1) as an eluent to give 20 fractions (*Fr.5a-5t*). *Fr.5f* was purified on a semiprep. HPLC column with MeCN/H₂O (53:47) as a mobile phase to yield **2** (2.9 mg) and **3** (2.5 mg). *Fr. 8* (10 g) was subjected to SiO₂ CC by using PE/acetone (1:1) as an eluent to give six fractions (*Fr.8a-8f*). *Fr.8b* was subsequently separated by prep. TLC and further purified on a semiprep. HPLC column with MeCN/H₂O (47:53) as a mobile phase to yield **1** (5 mg).

2,3-Dideacetylxyloccensin S (1). White powder. $[\alpha]_{\text{D}}^{24} = -20$ (*c*=0.010, CHCl₃). UV (CHCl₃): 214. IR (KBr): 3600-3210, 1740-1710. ¹H- and ¹³C-NMR (CDCl₃): see *Table 1*. HR-TOF-MS: 632.2109 (*M*⁺ calc. 632.2105).

30-Deacetylxyloccensin W (2). White powder. $[\alpha]_{\text{D}}^{24} = -45$ (*c*=0.010, CHCl₃). UV (CHCl₃): 214. IR (KBr): 3600-3210, 1740-1710. ¹H- and ¹³C-NMR (CDCl₃): see *Table 2*. HR-TOF-MS: 502.2208 (*M*⁺ calc. 502.2203).

7-Hydroxy-3-oxo-21β-methoxy-24,25,26,27-tetranortirucall-1,14-dien-23(21)-lactone (3)
White powder. $[\alpha]_{\text{D}}^{24} = -15$ (*c*=0.010, CHCl₃). IR (KBr): 3450, 1745-1715. ¹H- and ¹³C-NMR (CDCl₃): see *Table 3*. HR-TOF-MS: 442.2717 (*M*⁺ calc. 442.2719).

Acknowledgements

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Table 1 The NMR data for compound **1** in (CDCl₃)

Position	δ_{H} ; mult; $J(\text{Hz})$	δ_{C}	HMBC
1	--	84.1	
2	--	76.0	
3	3.71 (<i>d</i> , $J = 5.5$)	86.5	2
4	--	44.0	
5	2.36 (<i>br. s</i>)	44.4	
6	5.22 (<i>d</i> , $J = 0.9$)	70.9	
7	--	174.8	
8	--	84.0	
9	--	87.4	
10	--	47.9	
11a	2.32 (<i>dd</i> , $J = 13.6, 3.7$)	32.1	
11b	1.98-2.04 (<i>m</i>)		
12	4.82 (<i>dd</i> , $J = 13.6, 3.7$)	69.0	
13	--	42.6	
14	--	151.8	
15	6.58 (<i>s</i>)	123.7	8, 13
16	--	169.7	
17	5.78 (<i>s</i>)	78.6	20, 21, 22
18	1.57 (<i>s</i>)	14.0	12, 13, 14, 17
19	1.53 (<i>s</i>)	16.8	1, 5, 9, 10
20	--	121.2	
21	7.40 (<i>s</i>)	141.4	
22	6.53 (<i>d</i> , $J = 1.5$)	110.0	
23	7.40 (<i>d</i> , $J = 1.5$)	142.8	
28a	2.25 (<i>d</i> , $J = 12.7$)	39.8	
28b	1.62 (<i>br. s</i>)		
29	1.00 (<i>s</i>)	15.1	3, 28, 5
30	4.65 (<i>s</i>)	78.5	1, 9
1-OH	3.48 (<i>s</i>)		
2-OH	3.53 (<i>s</i>)		
3-OH	3.40 (<i>s</i>)		
6-OH	2.58 (<i>br. s</i>)	--	
7-OMe	3.84 (<i>s</i>)	52.6	7
Me-C(O ₃)	1.70 (<i>s</i>)	16.1/118.7	
12-Ac	1.53 (<i>s</i>)	19.6/170.3	

Table 2 The NMR data for compound **2** in (CDCl₃)

Position	δ_{H} ; mult; J (Hz)	δ_{C}	HMBC
1	--	213.1	
2	3.08 (<i>t</i> , $J = 6.3$)	53.4	1, 8, 30
3	4.15(<i>d</i> , $J = 5.9$)	87.3	1, 2, 5, 8, 28
4	--	37.2	
5	3.13 (br. <i>dd</i> , $J = 11.0, 2.1$)	43.1	
6	2.26 2.12	32.4	
7	--	174.1	
8	--	80.6	
9	2.37 (<i>dd</i> , $J = 13.0, 5.1$)	45.4	8, 10
10	--	50.4	
11a	2.19	20.5	
11b	1.57		
12a	1.75 (<i>td</i> , $J = 13.6, 3.7$)	28.5	
12b	1.49-1.55 (m)		
13	--	39.9	
14	--	75.8	
15a	3.17 (<i>d</i> , $J = 18.1$)	37.4	13, 16, 8, 14
15b	2.65 (<i>d</i> , $J = 18.1$)		8, 14, 16
16	--	169.6	
17	6.22 (<i>s</i>)	76.0	13, 18, 20, 21, 22
18	0.99 (<i>s</i>)	15.3	12, 13, 14, 17
19	0.98 (<i>s</i>)	16.2	1, 5, 9, 10
20	--	120.4	
21	7.58 (<i>s</i>)	140.7	22, 23
22	6.51 (<i>dd</i> , $J = 1.6, 0.8$)	109.8	20, 21
23	7.46 (<i>s</i>)	142.7	
28	1.09 (<i>s</i>)	27.1	3, 4, 5, 29
29	0.67 (<i>s</i>)	19.8	3,4, 5, 28
30	4.77 (<i>d</i> , $J = 6.8$)	77.4	1
7-OMe	3.71 (<i>s</i>)	51.7	7
14-OH	1.67 (<i>s</i>)		
30-OH	3.03 (<i>s</i>)	--	

Table 3 The NMR data for compound **3** in (CDCl₃)

Position	δ_H ; mult; J (Hz)	δ_C	HMBC
1	7.14 (<i>d</i> , $J = 10.2$)	157.7	
2	5.84 (<i>d</i> , $J = 10.2$)	125.3	
3	--	205.0	
4	--	36.4	
5	2.41 (<i>dd</i> , $J = 12.6, 2.7$)	44.5	
6	1.88-1.93 (<i>m</i>)	31.1	
7	3.16-3.22 (<i>m</i>)	71.3	
8	--	44.5	
9	2.28-2.35 (<i>m</i>)	36.4	
10	--	39.7	
11	1.58-1.65 (<i>m</i>)	17.8	
12	1.72-1.80 (<i>m</i>)	32.3	
13	--	46.5	
14	--	160.9	
15	5.52 (<i>dd</i> , $J = 9.7, 4.3$)	119.8	
16	1.45-1.50 (<i>m</i>)	27.2	
	2.15-2.23 (<i>m</i>)		
17	2.03-2.09 (<i>m</i>)	52.3	
18	1.04 (<i>s</i>)	19.9	12, 13, 14, 17
19	1.18 (<i>s</i>)	18.6	1, 5, 9, 10
20	2.38-2.45 (<i>m</i>)	43.9	
21	2.18-2.24 (<i>m</i>)	33.8	
22	--	175.4	
23	4.79 (<i>d</i> , $J = 4.2$)		
28	1.18 (<i>s</i>)	26.8	3, 4, 5, 29
29	1.11 (<i>s</i>)	21.2	3, 4, 5, 28
30	1.14 (<i>s</i>)	27.3	7, 8, 9, 14
7-OH	4.00 (br. <i>s</i>)		
23-OMe	3.39 (<i>s</i>)	54.8	

^a Multiplicity: s, singlet; d, doublet; t, triplet; m, multiplet; o, overlapped; and br, broad.

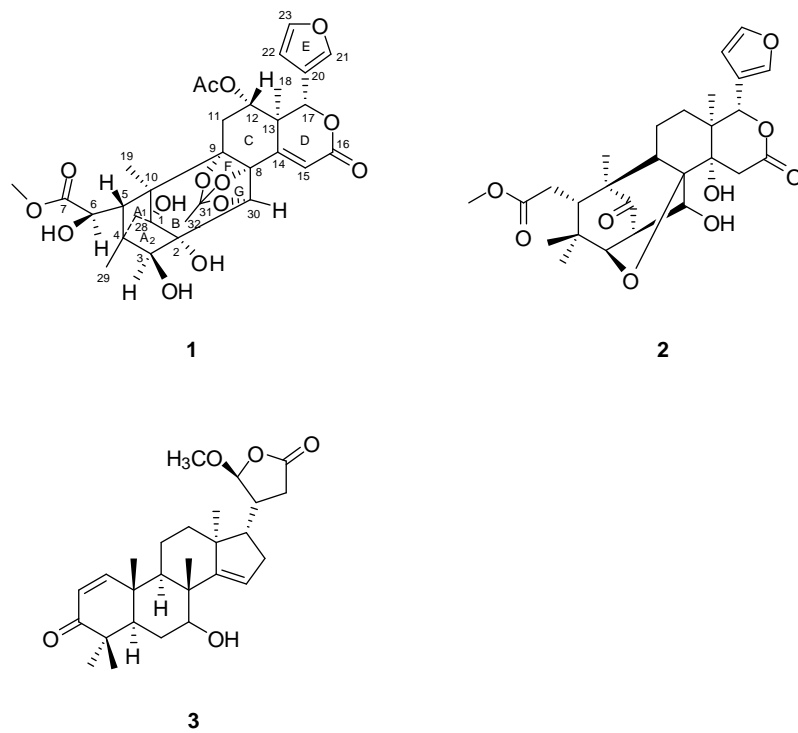


Figure 1. Structures of compounds (1-3)

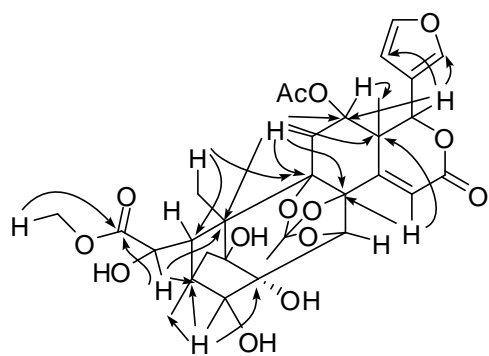


Figure 2. Key HMBCs of 1

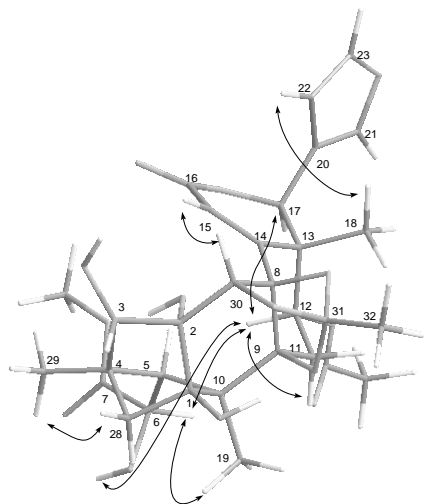


Figure 3. Calculated conformation by MM2 and significant NOESY correlations of **1**

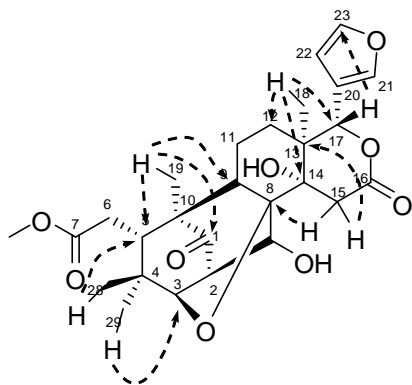


Figure 4. Key HMBCs of **2**.

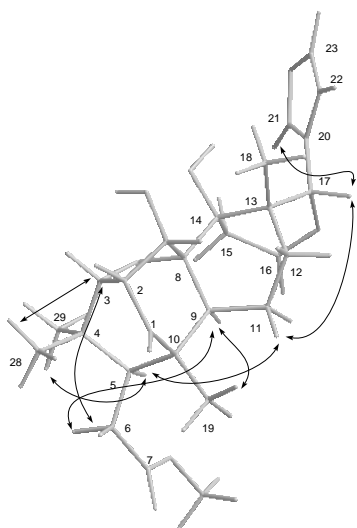


Figure 5. Calculated conformation by MM2 and significant NOESY correlations of **2**.

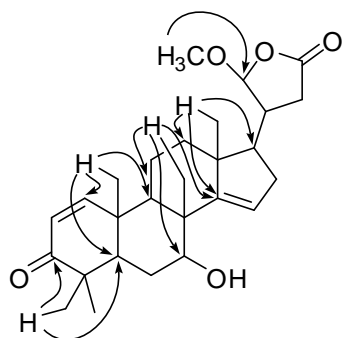


Figure 6. Key HMBCs of **3**.

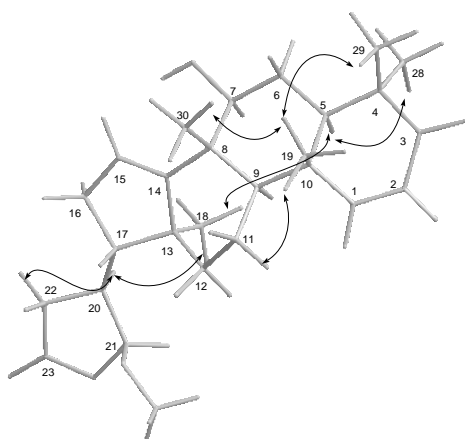


Figure 7. Calculated conformation by MM2 and significant NOESY correlations of **3**

Table 1 The NMR data for compound **1** in (CDCl₃)

Table 2 The NMR data for compound **2** in (CDCl₃)

Table 3 The NMR data for compound **3** in (CDCl₃)

Figure 1. Structures of compounds (**1-3**)

Figure 2. Key HMBCs of **1**

Figure 3. Calculated conformation by MM2 and significant NOESY correlations of **1**

Figure 4. Key HMBCs of **2**.

Figure 5. Calculated conformation by MM2 and significant NOESY correlations of **2**.

Figure 6. Key HMBCs of **3**.

Figure 7. Calculated conformation by MM2 and significant NOESY correlations of **3**