New Limonoids from the Seeds of *Xylocarpus granatum*

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Three novel limonoids, 2,3-dideacetylxyloccensin S (**1**), 30-deacetylxyloccensin W (**2**), and 7-hydroxy-21β-methoxy-3-oxo-24,25,26,27-tetranortirucalla-1,14-diene-23(21)-lactone (**3**), were isolated from the seeds of the Chinese mangrove, *Xylocarpus granatum*. The structures were elucidated on the basis of 1D- and 2D-NMR (including ^{1}H- and ^{13}C-NMR, DEPT, ^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY) data and confirmed by HR-MS.

Introduction. – *Xylocarpus granatum* J.KOENIG, a marine mangrove plant distributed mainly along the seashore of the Indian Ocean and in Southeast Asia, is used as a folk medicine in Southeast Asia for the treatment of diarrhea, cholera, and feverish 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 limonoids have been isolated from *X. granatum*, and they have been classified into phragmalin, mexicanolide, obacunol, and andirobin types [3–8].

Our previous investigations have resulted in the isolation and identification of three new limonoids from the seeds of a Chinese mangrove *X. granatum* [9][10]. Further investigation on the fruit of the same plant led to the discovery of further three novel compounds, 2,3-dideacetylxyloccensin S (**1**), 30-deacetylxyloccensin W (**2**), and 7-hydroxy-21β-methoxy-3-oxo-24,25,26,27-tetranortirucalla-1,14-diene-23(21)-lactone (**3**; *Fig. 1*). Herein, the isolation and structure elucidation of these three novel compounds are presented.

Results and Discussion. – 2,3-Dideacetylxyloccensin S (**1**) was obtained as white powder. The molecular formula was deduced as $C_{31}H_{36}O_{14}$ implying 14 degrees of unsaturation by HR-TOF-MS (*m*/*z* 632.2109 (M^+; calc. 632.2105)). The ^{13}C-NMR

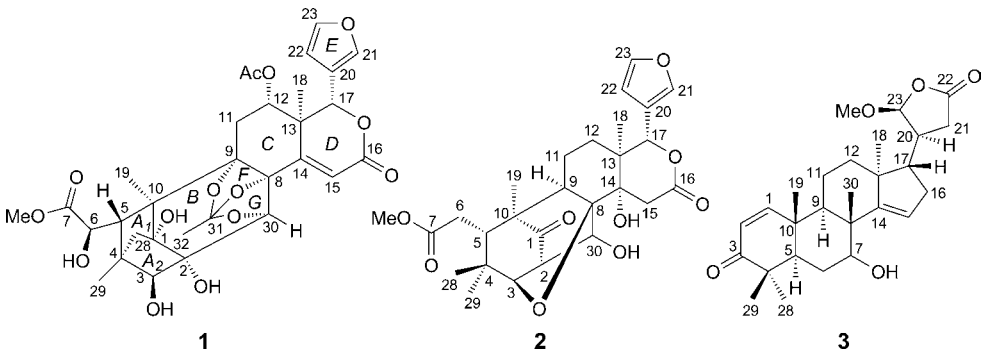

Fig. 1. *Structures of compounds* **1**–**3**

spectrum revealed that **1** contains six olefinic C-atoms and three C=O groups. Therefore, the remaining eight unsaturations indicated that **1** consisted eight rings. The ^{1}H- and ^{13}C-NMR spectra (*Table 1*) exhibited signals of six Me, two CH_2, ten CH groups (five O-bearing and four olefinic ones), and 13 quaternary C-atoms (four O-bearing, three ester, and two olefinic C-atoms). In addition, four OH groups (δ(H) 2.58 (br. *s*), 3.40 (*s*), 3.48 (br. s), and 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 furan ring (δ(H) 6.53 (*d*, $J = 1.5$), 7.40 (*d*, $J = 1.5$), and 7.40 (*s*); δ(C) 110.0, 142.8, 141.4, and 121.2) were assigned by means of the ^{1}H- and ^{13}C-NMR data. The aforementioned spectroscopic data implied that **1** was a type of phragmalin, consisting of eight rings, designated as A_1, A_2, and $B-G$. The structure of **1** was elucidated by analyses of the ^{1}H,^{1}H-COSY, HSQC, and HMBC data. The HMBCs 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 rings as depicted in *Fig. 2*. The HMBC cross-peaks from H–C(17) to C(20), C(21), and C(22); from Me(18) to C(13) and C(17); and from H–C(15) to C(8) and C(13), indicated the presence of the *C*, *D*, and *E* rings. The relative configuration of **1** was determined on the basis of the NOESY spectrum, and the three-dimensional drawing generated by MM2 calculation was shown in *Fig. 2*. H–C(17) exhibited a NOE with H–C(12), but not with Me(18); Me(18) displayed a NOE with H–C(22), indicating that the furan ring, Me(18), and 12-OH were on the same side. H–C(30) showed a NOE with H–C(15), suggesting that ring *D* was in a half-chair conformation. The NOE correlations H–C(6)/Me(19), and of CH_2(28)/Me(29) evidenced that Me(19) and HO–C(6) were on the opposite side, and the two five-carbocyclic rings (A_1 and A_2) adopted envelope conformations. Based on the above results, the relative configuration of **1** was elucidated as shown in *Fig. 2*. Xyloccensin S, 2,3-diacetyl derivative of **1**, had been isolated from this plant in 2005 [11].

30-Deacetylxyloccensin W (**2**) was obtained as white powder. The molecular formula was deduced as $C_{27}H_{34}O_9$, indicating eleven degrees of unsaturation, by HR-TOF-MS (*m*/*z* 502.2208 (M^+; calc. 502.2203)). The ^{13}C-NMR spectrum revealed that **2** contains four olefinic C-atoms and three C=O groups. Therefore, the remaining six unsaturations implied that **2** consisted six rings. The ^{1}H- and ^{13}C-NMR spectra

Table 1. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of Compound* **1**. Arbitrary atom numbering as indicated in *Fig. 1*; δ in ppm, J in Hz.

Position	δ(H)	δ(C)	HMBC
1	–	84.1	
2	–	76.0	
3	3.71 (*d*, $J = 5.5$)	86.5	2, 4
4	–	44.0	
5	2.36 (br. *s*)	44.4	
6	5.22 (*d*, $J = 0.9$)	70.9	
7	–	174.8	
8	–	84.0	
9	–	87.4	
10	–	47.9	
11	2.32 (*dd*, $J = 13.6$, 3.7, H_a), 1.98–2.04 (*m*, H_b)	32.1	
12	4.82 (*dd*, $J = 13.6$, 3.7)	69.0	
13	–	42.6	
14	–	151.8	
15	6.58 (*s*)	123.7	8, 13
16	–	169.7	
17	5.78 (*s*)	78.6	20, 21, 22
18	1.57 (*s*)	14.0	12, 13, 14, 17
19	1.53 (br. *s*)	16.8	1, 5, 9, 10
20	–	121.2	
21	7.40 (*s*)	141.4	
22	6.53 (*d*, $J = 1.5$)	110.0	
23	7.40 (*d*, $J = 1.5$)	142.8	
28	2.25 (*d*, $J = 12.7$, H_a), 1.62 (br. *s*, H_b)	39.8	
29	1.00 (*s*)	15.1	3, 4, 28, 5
30	4.65 (*s*)	78.5	1, 9
31	–	118.7	
1-OH	3.48 (br. *s*)		
2-OH	3.53 (*s*)		
3-OH	3.40 (*s*)		
6-OH	2.58 (br. *s*)	–	
7-MeO	3.84 (*s*)	52.6	7
Me(32)	1.70 (*s*)	16.1	
12-AcO	1.53 (*s*)	19.6, 170.3	

(*Table 2*) revealed the presence of five Me, four CH_2 and, nine CH groups (three O-bearing and three olefinic ones), and nine quaternary C-atoms (two O-bearing, two ester, and one olefinic). In addition, two OH groups (δ(H) 1.67 (*s*), 3.03 (br. *s*)), four tertiary Me groups (δ(H) 0.99 (*s*), 0.98 (*s*), 1.09 (*s*), and 0.67 (*s*); δ(C)15.3, 16.2, 27.1, and 19.8), one MeO group (δ(H) 3.71; δ(C) 51.7), and a β-substituted furan ring (δ(H) 6.51 (br. *dd*, $J = 1.6$, 0.8), 7.46 (*s*), and 7.58 (br. *s*); δ(C) 109.8, 142.7, 140.7, and 120.4) were assigned by the ^{1}H- and ^{13}C-NMR data. The structure of **2** was determined by analyses of the ^{1}H,^{1}H-COSY, HSQC, and HMBC data. The HMBCs 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 the *A* ring as shown in *Fig. 3*. The HMBC cross-peaks from H–C(17) to C(20), C(21), and C(22); from Me(18) to C(13) and C(17); from H_a–C(15)

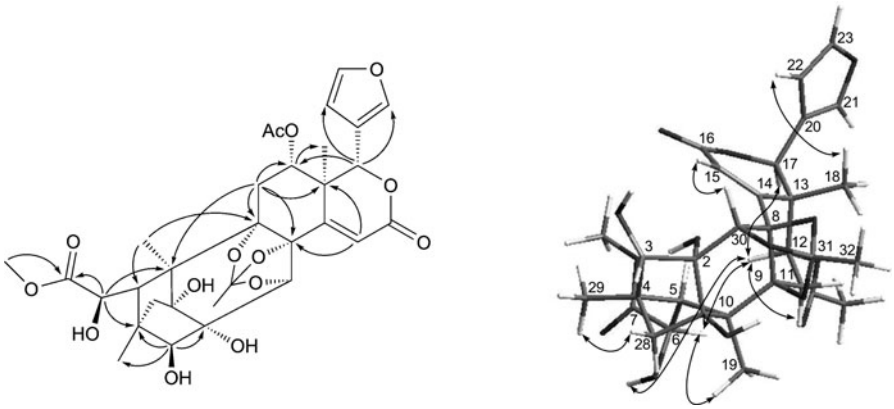

Fig. 2. *Key HMBCs* (H → C), *conformation calculated by MM2, and significant NOESY* (H ↔ H) *correlations of* **1**

Table 2. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of Compound* **2**. Arbitrary atom numbering as indicated in *Fig. 1*; δ in ppm, J in Hz.

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

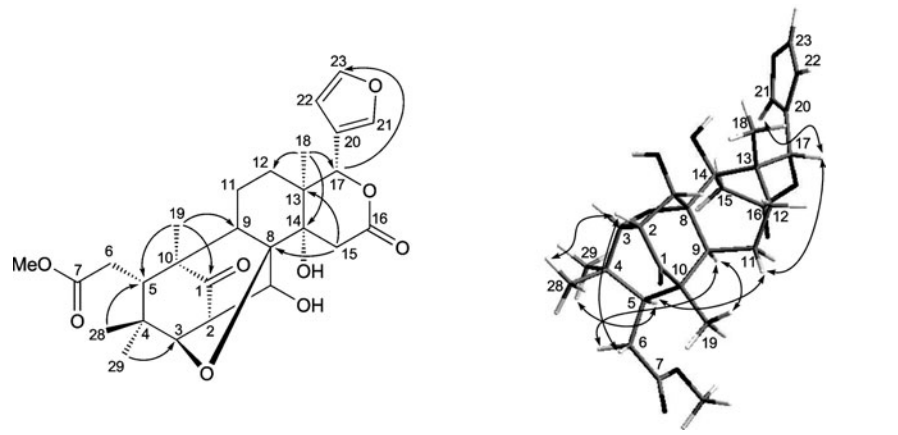

Fig. 3. *Key HMBCs* (H → C), *conformation calculated by MM2, and significant NOESY* (H ↔ H) *correlations of* **2**

to C(13), C(14), and C(16); and from H_b–C(15) to C(14) and C(8) indicated the positions of the *C*, *D*, and *E* rings. The relative configuration of **2** was determined 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 a NOE with H_a–C(11), but not with Me(18); Me(18) displayed a NOE with H–C(22), indicating that the furan ring and Me(18) were on the same side. The Me(19) exhibited a NOE with H–C(9), but not with H–C(5), evidencing that Me(19) and H–C(9) were on the same side, and Me(19) and H–C(5) on the opposite side. Based on the above results, the relative configuration of **2** was elucidated as shown in *Fig. 3*. Xyloccensin W, 30-acetyl derivative of **2**, had been isolated from this plant in 2006 [12].

7-Hydroxy-21β-methoxy-3-oxo-24,25,26,27-tetranortirucalla-1,14-diene-23(21)-lactone (**3**) was obtained as white powder. The molecular formula was deduced as $C_{27}H_{38}O_5$, *i.e.*, with nine degrees of unsaturation, by HR-TOF-MS (*m/z* 442.2717 (M^+; calc. 442.2719)). The ^{13}C-NMR spectrum revealed that **3** contained four olefinic C-atoms and two C=O groups. Therefore, the remaining five unsaturations were due to the presence of five rings. The ^{1}H- and ^{13}C-NMR spectra (*Table 3*) revealed the presence of six Me, five CH_2, and nine CH groups (two O-bearing and three olefinic ones), and seven quaternary C-atoms (one ester and one olefinic). 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) 19.9, 18.6, 27.3, 26.8, and 21.2), one MeO group (δ(H) 3.39; δ(C) 54.8), and a five-membered lactone (δ(H) 2.18–2.24 (*m*), 2.38–2.45 (*m*), and 4.79 (*d*, $J = 4.2$); δ(C) 33.8, 43.9, 104.5, and 175.4) were assigned by means of the ^{1}H- and ^{13}C-NMR data. The HMBCs Me(18)/C(12), Me(18)/C(14), Me(18)/C(17), Me(19)/C(1), Me(19)/C(5), Me(19)/C(9), Me(30)/C(7), Me(30)/C(9), Me(30)/C(14), Me(28)/C(3), Me(28)/C(5), and Me(28)/C(29) indicated that **3** was a typical tetracyclic tetranortriterpenoid with a five-membered lactone at C(17) (*Fig. 4*). The relative configuration of **3** was determined on the basis of the NOESY spectrum, and the three-dimensional drawing generated by MM2 calculation was shown in *Fig. 4*. The NOE correlations Me(28)/H–C(5), H–C(5)/Me(18), and Me(18)/H–C(20) evidenced that Me(28), H–C(5), and Me(18) were on

the same side. The NOE correlations of Me(19) with Me(30), Me(29), and H_β–C(11) indicated that the relative configuration of **3** was as shown in *Fig. 4*.

Table 3. *^{1}H- and ^{13}C-NMR* ($CDCl_3$) *Data of Compound* **3**. Arbitrary atom numbering as indicated in *Fig. 1*; δ in ppm, J in Hz.

Position	δ(H)	δ(C)	HMBC
1	7.14 (*d*, $J = 10.2$)	157.7	
2	5.84 (*d*, $J = 10.2$)	125.3	
3	–	205.0	
4	–	36.4	
5	2.41 (*dd*, $J = 12.6, 2.7$)	44.5	
6	1.88–1.93 (*m*)	31.1	
7	3.16–3.22 (*m*)	71.3	
8	–	44.5	
9	2.28–2.35 (*m*)	36.4	
10	–	39.7	
11	1.58–1.65 (*m*)	17.8	
12	1.72–1.80 (*m*)	32.3	
13	–	46.5	
14	–	160.9	
15	5.52 (*dd*, $J = 9.7, 4.3$)	119.8	
16	1.45–1.50 (*m*), 2.15–2.23 (*m*)	27.2	
17	2.03–2.09 (*m*)	52.3	
18	1.04 (*s*)	19.9	12, 13, 14, 17
19	1.18 (*s*)	18.6	1, 5, 9, 10
20	2.38–2.45 (*m*)	43.9	
21	2.18–2.24 (*m*)	33.8	
22	–	175.4	
23	4.79 (*d*, $J = 4.2$)	104.5	
28	1.18 (*s*)	26.8	3, 4, 5, 29
29	1.11 (*s*)	21.2	3, 4, 5, 28
30	1.14 (*s*)	27.3	7, 8, 9, 14
7-OH	4.00 (br. *s*)		
23-MeO	3.39 (*s*)	54.8	

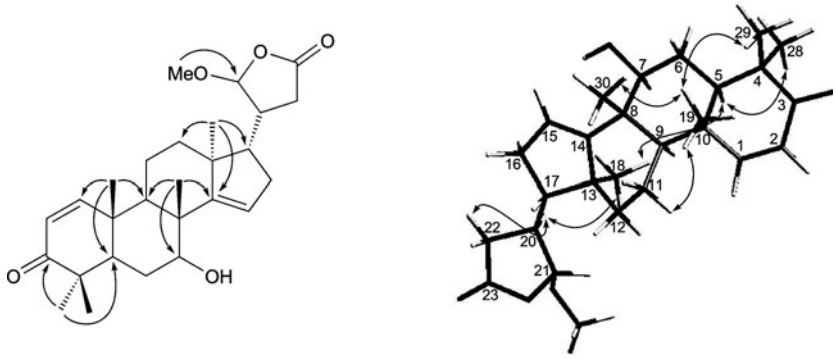

Fig. 4. *Key HMBCs* (H → C), *conformation calculated by MM2, and significant NOESY* (H ↔ H) *correlations of* **3**

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Factory*, P. R. China). Semi-prep. HPLC: *Waters Delta Prep 3000* pump, *UV 2487* detector, and *Whatman Partisil 10 ODS-2* column (9.4 × 250 mm). Optical rotation: *Jasco DIP-370*. NMR: *Bruker AV-600*; at 600.17 (^{1}H) and 150.93 MHz (^{13}C), in $CDCl_3$; δ in ppm rel. to Me_4Si as an internal standard; *J* in Hz. HR-TOF-MS: *Applied Biosystems QStar XL QqTOF*; in *m*/*z*.

Plant Material. Seeds of *X. granatum* were collected in March 2006 at Hainan Island, Southern China, dried at r.t., and identified by Dr. *Wen-Qing Wang* (School of Life Sciences, Xiamen University, P. R. China). Several voucher specimen (No. HEBNMC-2006-1) have been deposited with 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 r.t. After evaporation of the solvent under reduced pressure, the resulting residue was suspended in H_2O, and extracted with petroleum ether (PE) and CH_2Cl_2, successively. The CH_2Cl_2 extract (120 g) was subjected to CC (SiO_2; PE/AcOEt 30:1 to 1:10) to yield nine fractions, *Frs. 1–9*. *Fr. 5* (10 g) was seperated by CC (SiO_2; PE/acetone 3:1) to give 20 fractions, *Frs. 5a–5t*. *Fr. 5f* was purified by semi-prep. HPLC (MeCN/H_2O 53:47) to yield **2** (2.9 mg) and **3** (2.5 mg), resp. *Fr. 8* (10 g) was subjected to CC (SiO_2; PE/acetone 1:1) to give six fractions, *Frs. 8a–8f*. *Fr. 8b* was subsequently separated by prep. TLC and further purified by semi-prep. HPLC (MeCN/H_2O 47:53) to yield **1** (5 mg).

2,3-Dideacetylxyloccensin S (= *Methyl (*α*R,1S,4bR,7aR,8S,8aR,10R,11S,11aR,11bS,13S,13aS,15S)-13-(Acetyloxy)-6,11b-epoxy-1-(furan-3-yl)-3,7a,8,8a,10,11,11a,12,13,13a-decahydro-α,8,8a,15-tetrahydroxy-6,10,11a,13a-tetramethyl-3-oxo-9*H*-8,10-methano-1*H*-cyclopenta[5,6][1,3]dioxolo[8,8a]naphtho[2,1-c]pyran-11-acetate*; **1**). White powder. $[\alpha]_D^{24} = -20$ ($c = 0.010$, $CHCl_3$). UV ($CHCl_3$): 214. IR (KBr): 3600–3210, 1740–1710. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Table 1*. HR-TOF-MS: 632.2109 (M^+, $C_{31}H_{36}O_{14}^+$; calc. 632.2105).

30-Deacetylxyloccensin W (= *Methyl (4S,4aS,6aR,7S,8S,10R,12aR,12bS)-10,12a-Epoxy-4-(furan-3-yl)dodecahydro-12,12b-dihydroxy-4a,7,9,9-tetramethyl-2,14-dioxo-4*H*-7,11-methano-2*H*-cycloocta[3,4]benzo[1,2-c]pyran-8-aceate*; **2**). White powder. $[\alpha]_D^{24} = -45$ ($c = 0.010$, $CHCl_3$). UV ($CHCl_3$): 214. IR (KBr): 3600–3210, 1740–1710. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Table 2*. HR-TOF-MS: 502.2208 (M^+, $C_{27}H_{34}O_9^+$; calc. 502.2203).

*7-Hydroxy-21*β*-methoxy-3-oxo-24,25,26,27-tetranortirucalla-1,14-diene-23(21)-lactone* (= *(4S,5S)-4-[(5R,8R,9R,10R,13S,17S)-4,5,6,7,8,9,10,11,12,13,16,17-Dodecahydro-7-hydroxy-4,4,8,10,13-pentamethyl-3-oxo-3*H*-cyclopenta[a]phenanthren-17-yl]dihydro-5-methoxyfuran-2(3*H*)-one*; **3**). White powder. $[\alpha]_D^{24} = -15$ ($c = 0.010$, $CHCl_3$). IR (KBr): 3450, 1745–1715. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Table 3*. HR-TOF-MS: 442.2717 (M^+, $C_{27}H_{38}O_5^+$; calc. 442.2719).

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