Four New Eudesmane Sesquiterpenoid Lactones from *Chloranthus serratus*

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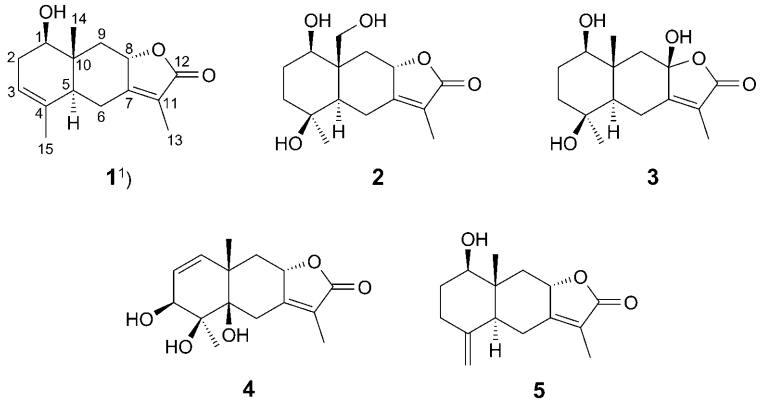
Four new eudesmane sesquiterpenoids endowed with an α,β-unsaturated γ-lactone five-membered ring, serralactones A–D (**1**–**4**, resp.), along with seven known sesquiterpenoids, were isolated from the whole plant *Chloranthus serratus*. Their structures and relative configurations were established on the basis of extensive spectroscopic analyses.

Introduction. – The genus *Chloranthus* (Chloranthaceae), which includes *ca.* 15 species, is mainly distributed in the east of Asia and has attracted considerable attention as a source of new sesquiterpenoids with diverse structures and biological properties [1][2]. *Chloranthus serratus* (THUNB.) ROEM. ET SCHULT., with the Chinese name 'Jiji', has long been used as treatment to activate blood circulation against stasis in Chinese folk medicine to cure injuries from falls, furuncle, tumefaction, and emmeniopathy [3]. Previous chemical investigations of this plant resulted in the isolation of various compounds including amides, sesquiterpenoids, and lindenane-type sesquiterpenoid dimers [4–10]. In our current investigation, four new eudesmane sesquiterpenoids with an α,β-unsaturated γ-lactone five-membered ring, serralactones A–D (**1**–**4**), and seven known sesquiterpenoids, neolitacumone B (**5**) [11], glechomanolide [12], zedoalactone A [13], oplodiol [14], cyperusol C [15], eudesm-4(15)-ene-1β,7,11-triol [16] and eudesm-3-ene-1β,7,11-triol [16] were isolated from the whole plants of *Chloranthus serratus*. The details of the isolation and structural elucidation of serralactones A–D (**1**–**4**, resp.), are discussed in this article.

Results and Discussion. – Serralactone A (**1**) was obtained as a white amorphous powder, and its molecular formula was established as $C_{15}H_{20}O_3$ by the HR-ESI-MS spectrum (*m*/*z* 249.1490, $[M+H]^+$; calc. 249.1490). The absorption bands in the IR spectrum at 1744 and 1681 cm^{-1}, and the UV maximum at 219 nm indicated the presence of an α,β-unsaturated γ-lactone moiety in **1** similar to neolitacumone B (**5**) [11]. The ^{1}H-NMR spectrum (*Table 1*) showed the presence of three Me *singlet*s at δ(H) 0.96, 1.71, 1.85 (Me(14), Me(15), and Me(13)[1]), resp.), and of one olefinic H-atom at δ(H) 5.40 (H–C(3)). The ^{13}C-NMR spectrum (*Table 2*) displayed 15 C-atom signals which were classified by a DEPT experiment into three Me, three CH_2, four CH

[1]) Arbitrary numbering. For systematic names, see *Exper. Part*.



groups (including two O-bearing and an olefinic CH group), and five quaternary C-atoms (including a lactone group and three olefinic C-atoms). Therefore, **1** was clearly recognized as an eudesm-7(11)-enolide sesquiterpene. Comprehensive analysis of NMR and mass spectra suggested that **1** was a C=C bond isomer of neolitacumone B (**5**) [11]. The position of the C(3)=C(4) bond was deduced from the HMBC correlations (*Fig.*) of δ(H) 3.57 (*dd*, H–C(1)) with δ(C) 32.3 (*t*, C(2)), 121.1 (*d*, C(3)), 47.3 (*d*, C(5)), 42.0 (*t*, C(9)), 38.9 (*s*, C(10)), and 10.0 (*q*, Me(14)), and δ(H) 5.40 (br. *s*, H–C(3)) with δ(C) 74.9 (*d*, C(1)), and 20.8 (*q*, C(15)), and δ(H) 1.71 (*s*, Me(15)) with δ(C) 121.1 (*d*, C(3)), 132.9 (*s*, C(4)), and 47.3 (*d*, C(5)). The relative configuration of **1** was established by a ROESY experiment, in which the correlations from Me(14) to H_β–C(6) and H–C(8) indicated that they were on the same side of the ring and were assigned being β-oriented. Consequently, the ROESY correlations of H_α–C(6)/H–C(5), and H–C(5)/H–C(1) suggested that H–C(1) and H–C(5) have α-orientation. Thus, compound **1** was identified as 1β-hydroxyeudesma-3,7(11)-dien-12,8α-olide.

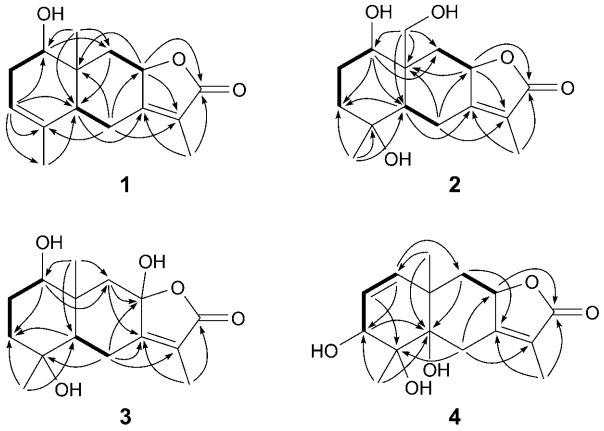


Figure. *^{1}H,^{1}H-COSY* (—) *and selected HMBC* (H → C) *of* **1**–**4**

Table 1. *^{1}H-NMR Data of Compounds* **1**–**4**[l]). δ in ppm, J in Hz.

	1[a])[c])	**2**[a])[c])	**3**[a])[c])	**3**[b])[c])	**4**[a])[d])	**4**[b])[d])
H–C(1)	3.57 (*dd*, J = 6.3, 10.1)	3.37 (*dd*, J = 4.4, 11.9)	3.19 (*dd*, J = 3.5, 11.6)	3.00–2.96 (*m*)	5.65 (*d*, J = 10.0)	5.54 (*d*, J = 10.0)
H_α–C(2)	2.37–2.33 (*m*)	1.69–1.65 (*m*)	1.53–1.49 (*m*)	1.32–1.29 (*m*)	5.88 (*dd*, J = 5.5, 10.0)	5.73 (*dd*, J = 5.4, 10.0)
H_β–C(2)	2.06–2.02 (*m*)	2.12–2.08 (*m*)	2.00–1.96 (*m*)	1.75–1.72 (*m*)		
H_α–C(3)	5.40 (br. *s*)	1.55–1.49 (*m*)	1.57–1.54 (*m*)	1.36–1.34 (*m*)	3.69 (*d*, J = 5.5)	3.56 (*t*, J = 5.4)
H_β–C(3)		1.74–1.70 (*m*)	1.76–1.72 (*m*)	1.55–1.52 (*m*)		
H–C(5)	2.00–1.97 (*m*)	1.31 (*dd*, J = 3.8, 13.5)	1.12 (*dd*, J = 2.6, 13.1)	0.93 (*dd*, J = 2.5, 12.9)		
H_α–C(6)	2.92 (*dd*, J = 3.9, 13.7)	2.91 (*dd*, J = 3.8, 13.5)	2.56–2.54 (*m*)	2.36–2.34 (*m*)	2.77 (*d*, J = 15.0)	2.66 (*d*, J = 11.6)
H_β–C(6)	2.21 (*t*, J = 13.7)	2.51 (*t*, J = 13.5)	2.81 (*dd*, J = 2.6, 13.1)	2.61 (*dd*, J = 2.5, 12.9)	2.91 (*d*, J = 15.0)	2.81 (*d*, J = 11.6)
H–C(8)	4.91 (*dd*, J = 6.8, 10.4)	5.00 (*dd*, J = 6.3, 11.8)		1.11–1.09 (*m*)	4.94 (*dd*, J = 6.4, 11.9)	5.04 (*dd*, J = 6.5, 12.0)
H_α–C(9)	1.01–0.99 (*m*)	0.78 (*t*, J = 11.8)	1.33–1.30 (*m*)	2.42–2.39 (*m*)	1.91 (*dd*, J = 6.4, 11.9)	1.80 (*dd*, J = 6.5, 12.0)
H_β–C(9)	2.75 (*dd*, J = 6.8, 10.4)	3.16 (*dd*, J = 6.3, 11.8)	2.60–2.58 (*m*)		2.16 (*t*, J = 11.9)	1.95 (*t*, J = 12.0)
Me(13)	1.85 (*s*)	1.80 (*s*)	1.81 (*s*)	1.68 (*s*)	1.81 (*s*)	1.69 (*s*)
Me(14)	0.96 (*s*)		1.31 (*s*)	1.14 (*s*)	1.36 (*s*)	1.21 (*s*)
H_a–C(14)		4.43 (*d*, J = 12.5)				
H_b–C(14)		3.76 (*d*, J = 12.5)				
Me(15)	1.71 (*s*)	1.21 (*s*)	1.25 (*s*)	1.09 (*s*)	1.19 (*s*)	1.11 (*s*)
1-OH				4.44 (*d*, J = 5.2)		
3-OH						5.45 (*d*, J = 5.6)
4-OH				4.04 (*s*)		4.75 (*s*)
5-OH						4.60 (*s*)
8-OH				7.01 (*s*)		

[a]) Recorded in (D_4)MeOH. [b]) Recorded in (D_6)DMSO. [c]) Recorded at 500 MHz. [d]) Recorded at 400 MHz.

Serralactone B (**2**), a white amorphous power, was shown to have the molecular formula $C_{15}H_{22}O_5$ on the basis of the HR-ESI-MS spectrum (*m*/*z* 305.1368, $[M+Na]^+$; calc. 305.1364). The ^{13}C-NMR (DEPT) spectrum (*Table 2*) showed 15 C-atom resonances assignable to a CO group (δ(C) 177.3), a tetrasubstituted C=C bond (δ(C) 120.7, 165.9), two Me, a HO–CH_2 (δ(C) 61.4), four CH_2, and two CH groups (including two O-bearing CH groups (δ(C) 79.9, 80.1)), as well as two quaternary C-atoms (including one O-bearing C-atom (δ(C) 71.1)). The data suggested that **2** is an eudesm-7(11)-enolide sesquiterpenoid possessing the same *B* and *C* rings as **1**, but a different *A* ring. The constitutional formula of **2** was determined on the basis of spectroscopic analyses and confirmed by ^{1}H,^{1}H-COSY and HMBC (*Fig.*). The fragment H–C(1)–CH_2(2)–CH_2(3)[1]) is positioned between C(10) and C(4) based on the observed ^{1}H,^{1}H-COSY correlations of H–C(2) with H–C(1) and H–C(3) and

Table 2. *^{13}C-NMR Data of Compounds* **1**–**4**[1]). δ in ppm.

	1[a])[c])	**2**[a])[c])	**3**[a])[c])	**3**[b])[c])	**4**[a])[d])	**4**[b])[d])
H–C(1)	74.9 (*d*)	80.1 (*d*)	80.1 (*d*)	77.8 (*d*)	133.5 (*d*)	134.0 (*d*)
CH_2(2) or H–C(2)	32.3 (*t*)	28.2 (*t*)	27.3 (*t*)	26.6 (*t*)	126.3 (*d*)	125.4 (*d*)
H–C(3) or CH_2(3)	121.1 (*d*)	39.9 (*t*)	40.1 (*t*)	39.9 (*t*)	72.1 (*d*)	71.3 (*d*)
C(4)	132.9 (*s*)	71.1 (*s*)	71.8 (*s*)	69.7 (*s*)	72.3 (*s*)	71.6 (*s*)
H–C(5) or C(5)	47.3 (*d*)	52.8 (*d*)	54.7 (*d*)	53.2 (*d*)	79.1 (*s*)	79.0 (*s*)
CH_2(6)	25.3 (*t*)	24.1 (*t*)	22.9 (*t*)	21.8 (*t*)	29.5 (*t*)	29.3 (*t*)
C(7)	162.0 (*s*)	165.9 (*s*)	164.1 (*s*)	162.8 (*s*)	161.7 (*s*)	163.4 (*s*)
H–C(8) or C(8)	78.5 (*d*)	79.9 (*d*)	105.7 (*s*)	104.2 (*s*)	78.5 (*d*)	78.0 (*d*)
CH_2(9)	42.0 (*t*)	41.5 (*t*)	51.4 (*t*)	50.4 (*t*)	39.2 (*t*)	39.0 (*t*)
C(10)	38.9 (*s*)	44.7 (*s*)	41.4 (*s*)	40.1 (*s*)	40.5 (*s*)	40.0 (*s*)
C(11)	120.6 (*s*)	120.7 (*s*)	121.9 (*s*)	119.7 (*s*)	122.7 (*s*)	120.6 (*s*)
C(12)	174.8 (*s*)	177.3 (*s*)	174.6 (*s*)	171.8 (*s*)	175.7 (*s*)	172.1 (*s*)
Me(13)	8.3 (*q*)	8.0 (*q*)	8.0 (*q*)	7.8 (*q*)	8.1 (*q*)	8.0 (*q*)
Me(14)	10.0 (*q*)	61.4 (*t*)	13.6 (*q*)	13.0 (*q*)	25.2 (*q*)	25.4 (*q*)
Me(15)	20.8 (*q*)	29.1 (*q*)	30.0 (*q*)	29.9 (*q*)	22.9 (*q*)	22.6 (*q*)

[a]) Recorded in (D_4)MeOH. [b]) Recorded in (D_6)DMSO. [c]) Recorded at 125 MHz. [d]) Recorded at 100 MHz.

the HMBC correlations of H–C(5) with C(1), C(3), C(4), C(7), C(9), C(10), and C(14). The HO–CH_2 group was placed at C(10) taking into account the HMBC correlations between CH_2(14) to C(1), C(5), C(9), and C(10). The relative configuration of **2** was deduced from the ROESY spectrum. The NOE interactions of CH_2(14) with H_β–C(2), H_β–C(6) and H–C(8), and of H–C(8) with H_β–C(9) suggested that they are placed on the same side of the molecule. Moreover, the cross peaks for H_α–C(2)/Me(15), H_α–C(9)/H–C(5), H–C(5)/H–C(1) indicated that H–C(1), H–C(5), and Me(15) lie on the opposite face. Hence, we have established that **2** is 1β,4β,14-trihydroxyeudesm-7(11)-en-12,8α-olide.

Serralactone C (**3**) was obtained as a white amorphous power. The HR-ESI-MS spectrum showed a *pseudo*-molecular ion peak at *m*/*z* 305.1373 ([M + Na]$^+$; calc. 305.1364) corresponding to the same molecular formula $C_{15}H_{22}O_5$ as that of compound **2**. The ^{1}H- and ^{13}C-NMR spectra of **3** (*Tables 1* and *2*) were similar to those of **2**, except for the absence of the HO–CH_2 group and the presence of a Me group at C(10)[1]) and a lactol group at C(8), as determined by the resonances at δ(H) 1.31 (*s*) and δ(C) 105.7 (*s*). In the HMBC spectrum (*Fig.*), the correlations from Me(14) to C(1), C(5), C(9), and C(10), and from both CH_2(6) and CH_2(9) to C(8) confirmed this deduction. The relative configuration of **3** was identical with the one of **2**, leaving open only C(8) of the lactol group. While it was impossible to determine the configuration of 8-OH in the lactol group due to the absence of ROESY correlations of the 8-OH signal and the other H-atoms resonance in (D_4)methanol, crucial NOE correlations between δ(H) 1.14 (*s*, Me(14)) and δ(H) 4.44 (*d*, 1-OH), 4.04 (*s*, 4-OH), and 7.01 (*s*, 8-OH) were observed in (D_6)DSMO, which revealed that they are positioned on the same side of the molecule. Hence, the structure of **3** is assumed to be 1β,4β,8β-trihydroxyeudesm-7(11)-en-12,8α-olide.

Serralactone D (**4**) was isolated as a white amorphous power with the molecular formula $C_{15}H_{20}O_5$ as deduced from the HR-ESI-MS spectrum (*m/z* 303.1202, $[M+Na]^+$; calc. 303.1208). The IR and UV spectra suggested that also **4** contained an α,β-unsaturated γ-lactone moiety. The ^{1}H- and ^{13}C-NMR spectra of **4** (*Tables 1* and *2*) displayed a CO group, a disubstituted CH = CH group, a tetrasubstituted C=C bond, three Me, two CH_2, and two O-bearing CH groups, as well as three quaternary C-atoms (including two O-bearing ones). These informations pointed to **4** being an eudesm-7(11)-enolide sesquiterpene possessing the same *B* and *C* rings as **1** and **2**, but a different *A* ring. The disubstituted CH=CH group was assigned to be placed between C(1) and C(2)[1]) based on the long-range correlations of H–C(1) with C(5), C(9), C(10) and Me(14), of H–C(2) with C(1) and C(10) in the HMBC spectrum (*Fig.*). Due to the presence of the ^{1}H,^{1}H-COSY correlation (*Fig.*) of H–C(3)/H–C(2) and the HMBC correlations of H–C(1)/C(3), H–C(1)/C(5), H–C(2)/C(4), Me(14)/C(5), Me(15)/C(3), Me(15)/C(4) and Me(15)/C(5), the three OH groups were placed at C(3), C(4), and C(5), respectively. The relative configuration of **4** was determined on the basis of ROESY data. The ROESY interactions of Me(14) with H_β–C(6), H–C(8), and H_β–C(9) suggested that they are placed on the same side of the molecule. Moreover, the ROESY correlations for H_α–C(6)/Me(15) and for Me(15)/H–C(3) disclosed that both H–C(3) and Me(15) lie on the opposite face. In order to determine the relative configuration of 5-OH, NMR experiments were reperformed in (D_6)DMSO. Key ROESY correlations of δ(H) 1.21 (*s*, Me(14)) with 5.45 (*d*, 3-OH), 4.75 (*s*, 4-OH), and 4.60 (*s*, 5-OH) were observed, which implied that they were placed on the β side. Therefore, compound **4** was established as 3β,4β,5β-trihydroxyeudesma-1,7(11)-dien-12,8α-olide.

Experimental Part

General. Semi-preparative HPLC: *Agilent 1100* apparatus; *Zorbax SB-C-18* column (*Agilent*, 9.4 mm × 25 cm). Column chromatography (CC): on silica gel (SiO_2; 200–300 mesh, *Qingdao Marine Chemical Inc*, P. R. China), or on silica gel *H* (10–40 μm, *Qingdao Marine Chemical Inc.*), or *Sephadex LH-20* (*Pharmacia*). Fractions were monitored by TLC and spots were visualized by heating the silica gel plates sprayed with 10% H_2SO_4 in EtOH. $[\alpha]_D$: *JASCO DIP-370* digital polarimeter. UV Spectra: *Shimadzu UV-2401 PC* spectrophotometer. IR Spectra: *Bio-Rad FTS-135* spectrometer, KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AM-400* instrument (400/100 MHz) and *Bruker DRX-500* instrument (500/125 MHz); δ in ppm rel. to TMS as internal standard, *J* in Hz. EI-MS: *VG Auto Spec-3000* mass spectrometer; in *m/z*. HR-ESI-MS: *API Qstar Pulsar LC/TOF* instrument.

Plant Material. The whole plants of *C. serratus* were collected in July 2007 from Xinning, Hunan Province, P. R. China, and identified by Dr. *Jian-Ying Xiang* of Kunming Institute of Botany. A voucher specimen (No. 200702) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China.

Extraction and Isolation. Dried and powdered whole plants of *C. serratus* (8 kg) were extracted with 70% acetone/H_2O (5 × 50 l) at r.t. The extract was concentrated under reduced pressure to obtain a dark crude extract (200 g), which was then subjected to a column of *D-941* gel eluted with 70% and 90% aq. EtOH. The evaporated 70% EtOH fraction was separated on a SiO_2 column eluted with petroleum ether (PE)/AcOEt (10:1–1:1) to yield eight fractions (*Frs. I–VIII*). *Fr. I* (10.0 g) was repeatedly chromatographed over SiO_2 (PE/acetone 10:1), and semi-preparative HPLC (MeCN/H_2O 30:70) to afford **1** (10 mg), and zedoalactone A (21 mg). *Fr. V* (5.8 g) was repeatedly separated on SiO_2 using $CHCl_3$/MeOH (100:1) to afford **2** (8 mg), **3** (9 mg), **4** (13 mg), and **5** (42 mg). *Fr. VI* (7.0 g) was subjected to SiO_2 column using $CHCl_3$/MeOH (90:1) as eluent, then purified on *Sephadex LH-20* (MeOH) to yield

glechomanolide (8 mg), oplodiol (35 mg), and cyperusol C (28 mg). *Fr. VII* was first chromatographed over SiO_2 using $CHCl_3$/MeOH (85 :1) as eluent, and then purified on semi-preparative HPLC (MeCN/H_2O, 30 :70; flow rate: 3 ml/min; UV detector at 220 nm) to give eudesm-4(15)-ene-1β,7,11-triol (42 mg), and eudesm-3-ene-1β,7,11-triol (15 mg).

Serralactone A (=*1β-Hydroxyeudesma-3(4),7(11)-dien-12,8α-olide* = *(4a*S*,8*R*,8a*R*,9a*S*)-4a,7,8,8a,9,9a-Hexahydro-8-hydroxy-3,5,8a-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one*; **1**). White amorphous power. $[\alpha]_D^{21} = +31.91$ ($c = 0.09$, MeOH). UV (MeOH): 219. IR (KBr): 3435, 2924, 1744, 1681, 1034. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 287 (13, $[M+K]^+$). HR-ESI-MS: 249.1490 ($[M+H]^+$, $C_{15}H_{21}O_3^+$; calc. 249.1490).

Serralactone B (=*1β,4β,14-Trihydroxyeudesm-7(11)-en-12,8α-olide* = *(4a*R*,5*S*,8*R*,8a*R*,9a*S*)-4a,5,6,7,8,8a,9,9a-Octahydro-5,8-dihydroxy-8a-(hydroxymethyl)-3,5-dimethylnaphtho[2,3-*b*]furan-2(4*H*)-one*; **2**). White amorphous power. $[\alpha]_D^{22} = +17.68$ ($c = 0.07$, MeOH). UV (MeOH): 220. IR (KBr): 3429, 2930, 1744, 1679, 1036. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 282 (1, M^+), 264 (12, $[M-H_2O]^+$), 246 (36, $[M-2\ H_2O]^+$), 228 (37, $[M-3\ H_2O]^+$), 216 (100), 188 (20), 160 (35), 145 (26), 105 (23), 91 (26), 77 (19). HR-ESI-MS: 305.1368 ($[M+Na]^+$, $C_{15}H_{22}NaO_5^+$; calc. 305.1364).

Serralactone C (=*1β,4β,8β-Trihydroxyeudesm-7(11)-en-12,8α-olide* = *(4a*R*,5*S*,8*R*,8a*R*,9a*S*)-4a,5,6,7,8,8a,9,9a-Octahydro-5,8,9a-trihydroxy-3,5,8a-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one*; **3**). White amorphous power. $[\alpha]_D^{21} = +0.0$ ($c = 0.07$, MeOH). UV (MeOH): 221. IR (KBr): 3417, 2946, 1737, 1689, 1242, 1142. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 282 (2, M^+), 264 (19, $[M-H_2O]^+$), 246 (40, $[M-2\ H_2O]^+$), 218 (100), 203 (32), 173 (66), 161 (52), 147 (35), 135 (36), 121 (41), 107 (26), 91 (38), 77 (25). HR-ESI-MS: 305.1373 ($[M+Na]^+$, $C_{15}H_{22}NaO_5^+$; calc. 305.1364).

Serralactone D (=*3β,4β,5β-Trihydroxyeudesma-1,7(11)-dien-12,8α-olide* = *(4a*R*,5*S*,6*S*,8a*S*,9a*S*)-4a,5,6,8a,9,9a-Hexahydro-4a,5,6-trihydroxy-3,5,8a-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one*; **4**). White amorphous power. $[\alpha]_D^{20} = +145.16$ ($c = 0.06$, MeOH). UV (MeOH): 220. IR (KBr): 3429, 2929, 1732, 1684, 1041. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 280 (1, M^+), 264 (8, $[M-H_2O]^+$), 244 (6, $[M-2\ H_2O]^+$), 234 (100), 216 (29), 201 (19), 173 (27), 163 (24), 145 (28), 123 (52), 100 (36), 91 (18), 77 (15). HR-ESI-MS: 303.1202 ($[M+Na]^+$, $C_{15}H_{20}NaO_5^+$; calc. 303.1208).

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