Nordammarane Triterpenoids from *Sanguisorba officinalis*

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Three new nordammarane triterpenoids, 12*β*-*O*-acetyl-3*β*,22-dihydroxy-23,24,25,26,27-pentanordammarane (**1**), 12*β*,22-dihydroxy-3-oxo-23,24,25,26,27-pentanordammarane (**2**), and 3*β*,12*β*-dihydroxy-23,24,25,26,27-pentanordammarane-22-carbaldehyde (**3**), were isolated from the EtOH extract of the roots of *Sanguisorba officinalis*. Structural elucidation of these new triterpenoids on the basis of spectroscopic analyses, including including 1D- and 2D-NMR, and HR-ESI-MS, is reported

Introduction. – The dried root of *Sanguisorba officinalis* L. (Rosaceae) is a traditionally valuable plant with hemostatic, analgesic, and astringent properties [1][2]. In Korea, Japan, and China, this plant has been used for the treatment of inflammatory and metabolic diseases such as diarrhea, chronic intestinal infections, duodenal ulcers, and internal hemorrhage [3][4]. Recent investigations have confirmed its anti-inflammatory, anticancer, antiallergic, anti-wrinkle, neuroprotective, and anxiolytic activities by evaluating the cellular mechanisms [5–7]. So far, saponin components such as triterpenes and their glycosides (*e.g.*, ziyuglycoside I), gallic acid, and a disaccharide (5-*O*-*α*-D-[3-*C*-(hydroxymethyl)lyxofuranosyl-*β*-D-[2-*C*-(hydroxymethyl)]arabinofuranose) have been reported as major active principles of the roots of *S. officinalis*, and they are considered to be responsible for the *in vitro* and *in vivo* pharmacological effects of this crude drug [7–10]. In continuation of our search for pharmacologically and structurally interesting substances from traditional Chinese medicines, the present study was undertaken to systematically examined, the triterpenoids from the EtOH extract of the roots of *S. officinalis*, and led to the isolation of three new nordammarane triterpenoids, *i.e.*, 12*β*-*O*-acetyl-3*β*,22-dihydroxy-23,24,25,26,27-pentanordammarane (**1**), 12*β*,22-dihydroxy-3-oxo-23,24,25,26,27-pentanordammarane (**2**), and 3*β*,12*β*-dihydroxy-23,24,25,26,27-pentanordammarane-22-carbaldehyde (**3**; *Fig. 1*). Their structures were elucidated by NMR spectroscopic and mass-spectroscopic methods.

Results and Discussion. – The AcOEt fraction of the EtOH extract of *S. officinalis* was purified by repeated column chromatography to afford compounds **1**, **2**, and **3**. Their structures were elucidated on the basis of the physical and spectroscopic data, including those by 1D- and 2D-NMR techniques.

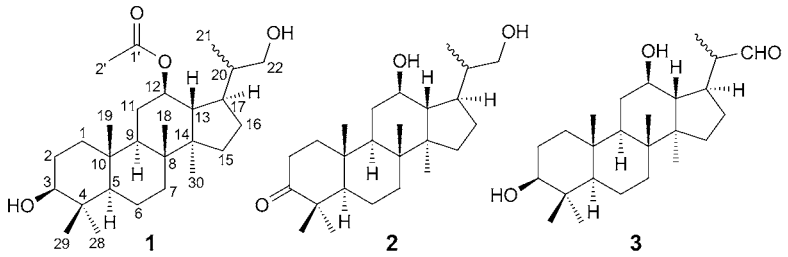

Fig. 1. *Structures of compounds* **1**–**3**

Compound **1** was obtained as white amorphous powder, and its molecular formula was deduced as $C_{27}H_{46}O_4$ from HR-ESI-MS (*m*/*z* 457.3290 ($[M+Na]^+$; calc. 457.3294)) and ^{13}C-NMR analyses (*Table 1*), corresponding to five unsaturation degrees. The IR of **1** showed strong absorptions of OH (3535 cm^{-1}) and AcO groups (1740 cm^{-1}), which was further supported by the NMR data (δ(C) 170.3 (*s*) and 21.2 (*q*); δ(H) 2.01 (*s*)). In the ^{1}H-NMR of **1** (*Table 2*), five Me *singlet*s, one Me *doublet*, signals of two O-bearing CH groups (δ(H) 3.17 (*dd*, J = 13.8, 3.6, 1 H) and 3.49 (*ddd*, J = 13.8, 13.2, 3.6, 1 H)),

Table 1. *^{13}C-NMR Data of Compounds* **1**–**3**. In (D_5)Pyridine; δ in ppm.

Position	**1**	**2**	**3**
1	39.2 (*t*)	39.7 (*t*)	38.8 (*t*)
2	27.2 (*t*)	33.9 (*t*)	27.5 (*t*)
3	79.0 (*d*)	218.6 (*s*)	78.8 (*d*)
4	38.9 (*s*)	47.3 (*s*)	38.6 (*s*)
5	55.8 (*d*)	55.2 (*d*)	55.1 (*d*)
6	18.3 (*t*)	19.6 (*t*)	18.7 (*t*)
7	35.6 (*t*)	34.3 (*t*)	35.0 (*t*)
8	40.5 (*s*)	39.8 (*s*)	40.9 (*s*)
9	50.8 (*d*)	49.6 (*d*)	51.3 (*d*)
10	37.0 (*s*)	36.8 (*s*)	37.3 (*s*)
11	32.3 (*t*)	32.5 (*t*)	32.0 (*t*)
12	73.3 (*d*)	71.2 (*d*)	71.0 (*d*)
13	47.4 (*d*)	47.8 (*d*)	47.8 (*d*)
14	51.6 (*s*)	51.5 (*s*)	51.0 (*s*)
15	31.6 (*t*)	31.3 (*t*)	31.8 (*t*)
16	26.0 (*t*)	26.4 (*t*)	26.2 (*t*)
17	52.3 (*d*)	51.9 (*d*)	52.0 (*d*)
18	15.4 (*q*)	16.0(*q*)	16.1 (*q*)
19	15.7 (*q*)	15.2 (*q*)	15.2 (*q*)
20	36.2 (*d*)	36.3 (*d*)	43.2 (*d*)
21	30.2 (*q*)	29.4 (*q*)	29.8 (*q*)
22	65.7 (*t*)	65.5 (*t*)	195.3 (*d*)
28	28.1 (*q*)	26.6 (*q*)	27.9 (*q*)
29	16.2 (*q*)	16.9 (*q*)	17.3 (*q*)
30	15.9(*q*)	16.8 (*q*)	16.6 (*q*)
MeC=O	170.3 (*s*),	–	–
	21.2 (*q*)	–	–

Table 2. *^{1}H-NMR Data of Compounds* **1**–**3**. *I*n (D_5)Pyridine; δ in ppm, *J* in Hz.

H-atom	**1**	**2**	**3**
H_{ax}–C(1)	1.20 (*ddd*, *J* = 13.8, 13.2, 3.6)	1.46–1.50 (overlapped)	1.19 (*ddd*, *J* = 13.6, 13.0, 3.8)
H_{eq}–C(1)	1.70–1.75 (overlapped)	1.95 (*ddd*, *J* = 13.2, 3.8, 3.2)	1.72 (*ddd*, *J* = 13.0, 3.6, 3.2)
H_{ax}–C(2)	1.65–1.67 (overlapped)	2.42 (*ddd*, *J* = 13.6, 13.0, 3.8)	1.61–1.66 (overlapped)
H_{eq}–C(2)	1.90–1.95 (overlapped)	2.52 (*ddd*, *J* = 13.0, 3.6, 3.2)	1.88–1.92 (overlapped)
H_{ax}–C(3)	3.17 (*dd*, *J* = 13.8, 3.6)	–	3.19 (*dd*, *J* = 13.6, 3.6)
H–C(5)	1.39 (*dd*, *J* = 13.8, 3.6)	1.35–1.40 (overlapped)	1.32–1.36 (overlapped)
H_{ax}–C(6)	1.42–1.46 (*m*)	1.41–1.45 (*m*)	1.37–1.40 (*m*)
H_{eq}–C(6)	1.60–1.63 (*m*)	1.57–1.61 (*m*)	1.57–1.60 (*m*)
H_{ax}–C(7)	1.28–1.34 (overlapped)	1.35–1.40 (overlapped)	1.32–1.36 (overlapped)
H_{eq}–C(7)	1.47–1.51 (overlapped)	1.46–1.50 (overlapped)	1.46 (*ddd*, *J* = 13.6, 3.2, 3.0)
H–C(9)	1.26 (*dd*, *J* = 13.6, 3.2)	1.53 (*dd*, *J* = 13.8, 3.6)	1.24 (*dd*, *J* = 13.8, 3.8)
H_{ax}–C(11)	1.28–1.34 (overlapped)	1.27 (*ddd*, 13.8, 13.6, 13.2)	1.32–1.36 (overlapped)
H_{eq}–C(11)	1.90–1.95 (overlapped)	1.85–1.90 (overlapped)	1.88–1.92 (overlapped)
H–C(12)	3.49 (*ddd*, *J* = 13.8, 13.2, 3.6)	3.51 (*ddd*, *J* = 13.8, 13.2, 3.6)	3.47 (*ddd*, *J* = 13.6, 13.2, 3.6)
H–C(13)	2.05 (*dd*, *J* = 13.8, 13.2)	2.09 (*dd*, *J* = 13.8, 13.6)	2.02 (*dd*, *J* = 13.6, 13.2)
H_{ax}–C(15)	1.15 (*ddd*, *J* = 13.8, 13.2, 3.2)	1.19 (*ddd*, *J* = 13.8, 13.2, 3.6)	1.11–1.16 (overlapped)
H_{eq}–C(15)	1.65–1.67 (overlapped)	1.68 (*ddd*, *J* = 13.2, 3.8, 3.2)	1.61–1.66 (overlapped)
H_{ax}–C(16)	1.70–1.75 (overlapped)	1.72–1.76 (*m*)	1.74–1.77 (*m*)
H_{eq}–C(16)	1.90–1.95 (overlapped)	1.85–1.90 (overlapped)	1.93–1.99 (overlapped)
H–C(17)	1.90–1.95 (overlapped)	1.92 (*ddd*, *J* = 13.8, 13.6, 3.6)	1.88–1.92 (overlapped)
Me(18)	0.98 (*s*)	0.99 (*s*)	0.95 (*s*)
Me(19)	1.07 (*s*)	1.06 (*s*)	1.05 (*s*)
H–C(20)	2.00–2.03 (*m*)	2.02–2.04 (*m*)	1.93–1.99 (overlapped)
Me(21)	1.06 (*d*, *J* = 6.8)	1.08 (*d*, *J* = 7.0)	1.11–1.16 (overlapped)
H_a–C(22)	3.36 (*dd*, *J* = 14.0, 6.8)	3.31 (*dd*, *J* = 13.8, 7.0)	9.68 (*d*, *J* = 6.8)
H_b–C(22)	3.62 (*dd*, *J* = 14.0, 6.8)	3.58 (*dd*, *J* = 13.8, 7.0)	–
Me(28)	1.07 (*s*)	1.09 (*s*)	1.03 (*s*)
Me(29)	1.01 (*s*)	1.04 (*s*)	0.98 (*s*)
Me(30)	0.87 (*s*)	0.91(*s*)	0.85 (*s*)
MeC=O	2.01 (*s*)	–	–

as well as of one O-bearing CH_2 group (δ(H) 3.36 and 3.62 (each *d*, *J* = 14.0, 6.8, 1 H)) were observed. The ^{13}C-NMR and DEPT spectra of **1** revealed the presence of five quaternary C-atoms (including one ester, and four sp^3-C-atoms), seven CH groups (including two O-bearing C-atoms), and eight CH_2 and seven Me groups. Apart from the AcO group, the remaining elements of the unsaturation in **1** were assumed to be due to the presence of four rings. Taking into account the characteristic of ^{13}C- and ^{1}H-NMR data, and the molecular formula, compound **1** was a nordammarane triterpenoid and should possess the skeleton of 23,24,25,26,27-pentanordammarane [11][12]. The presence two O-bearing CH (δ(C) 79.0 and 73.3) and one O-bearing CH_2 group (δ(C) 65.7) were deduced from AcO and OH substituents whose positions were determined by ^{1}H,^{1}H-COSY and HMBC experiments (*Fig. 2*). Three spin systems were identified as CH_2(1)/CH_2(2)/H–C(3)–O–, H–C(9)/CH_2(11)/H–C(12)–O–/H–C(13), and CH_2(16)/H–C(17)/H–C(20)/CH_2(22)–O– in the ^{1}H,^{1}H-COSY spectrum, and HMBCs H–C(12)/C(1′) (δ(C) 170.3) of Ac; H–C(5)/Me(28), Me(29), and C(3) (δ(C) 79.0); and of H–C(17)/(21) and C(22) (δ(C) 65.7), indicated that the AcO and the two OH groups were located at C(12), and C(3) and C(22) respectively.

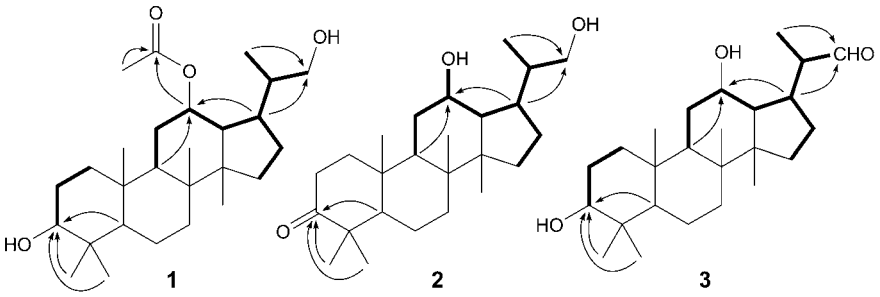

Fig. 2. *Key HMBC* (→) *and* $^1H,^1H$*-COSY* (—) *correlations of compounds* **1**–**3**

The configuration of **1** was determined by NOESY experiment (*Fig. 2*). The NOESY correlations H_α–C(1)/H–C(5)/H–C(9), H–C(9)/Me(30), H–C(17)/Me(30), H_β–C(1)/Me(18)/Me(19), and H_β–C(11)/H–C(13)/Me(18) indicated α-orientation for H–C(5), H–C(9), H–C(17), and Me(30), while H–C(13), Me(18), and Me(19) were β-oriented. In NOESY spectrum, the correlations between H–C(12) with H–C(9) and Me(30) evidenced β-orientation of AcO. In addition, correlations H–C(3) with H–C(5) and Me(28) indicated β-orientation for OH at C(3). Therefore, the structure of **1** was elucidated as 12β-*O*-acetyl-3β,22-dihydroxy-23,24,25,26,27-pentanordammarane.

Compound **2**, white amorphous powder, had the molecular formula $C_{25}H_{42}O_3$ as deduced from its HR-ESI-MS (*m*/*z* 413.3035 ($[M+Na]^+$; calc. 413.3032)). Its IR spectrum showed absorbtions of OH (3438 cm^{-1}) and C=O (1715 cm^{-1}) groups. The NMR data of compounds **1** and **2** (*Tables 1* and *2*) were similar in structure, except indicating that a OH group replaced the AcO group at C(12) in **1**, and the H- and C-atom signals of the O-bearing C(3)H group in **1** were replaced by those of a ketone (δ(C) 218.6) in **2**, which was further confirmed by the HMBC between H–C(5), Me(28), and Me(29) with C(3) (*Fig. 2*). On the basis of the similarity of NOESY data to those of **1**, the configuration of **2** was assumed to be the same. The NOESY correlations H–C(12)/H–C(9) and Me(30) indicated that OH at C(12) was β-oriented. Accordingly, the structure of **2** was established as 12β,22-dihydroxy-3-oxo-23,24,25,26,27-pentanordammarane.

Compound **3**, white amorphous powder, was assigned the molecular formula $C_{25}H_{42}O_3$ on the basis of its HR-ESI-MS (*m*/*z* 413.3028 ($[M+Na]^+$, $C_{25}H_{42}NaO_3^+$; calc. 413.3032)). Strong broad absorption at 3451 and 1735 cm^{-1} in the IR spectrum of **3** indicated the presence of OH and CHO groups, respectively. The general features of its IR and NMR spectra (*Tables 1* and *2*) closely resembled those of **1**, except indicating the replacement of the AcO group at C(12) in **1** by a OH group in **3**. Further, the signals of an aldehyde group (δ(C) 195.3 (*d*); δ(H) 9.68 (*d*, $J = 6.8$)) in **3** apeared in place of those of the CH_2(22) group in **1**, which was supported by the spin system of CH_2(16)/H–C(17)/H–C(20)/H–C(22)–O in the $^1H,^1H$-COSY spectrum and HMBCs of H–C(17) and H–C(21) with C(22) (δ(C) 195.3) (*Fig. 2*). The relative configuration of **3** was the same as that of **1** as established by the NOESY spectrum. All these data for **3** were consistent with the structure of 3β,12β-dihydroxy-23,24,25,26,27-pentanordammarane-22-carbaldehyde.

Experimental Part

1. *General.* Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, 10–40 μm; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China), *Sephadex LH-20* (*Amersham Pharmacia Biotech*, Sweden), *MCI Gel CHP20P* (75–150 μm; *Mitsubishi Kasei Chemical Industries*), and C_{18} reversed-phase (RP) silica gel (20–45 μm; *Fuji Silysia Chemical Ltd.*). TLC: Silica gel GF_{254} (10–40 μm; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China). All solvents were distilled before use. HPLC (anal. and prep.): *Shimadzu* model *LC-8A* on *YMC-pack*, *R&D ODS* column (250 × 4.6 mm, 250 × 20 mm) and UV detector *Shimadzu SPD-10AVP.* M.p.: *Tempo* melting-point apparatus; uncorrected. Optical rotations: *JASCO-20C* digital polarimeter. UV Spectra: *Hewlett-Packard-8452A* diode-array spectrophotometer. IR Spectra: *Perkin-Elmer 577* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker AM-400* spectrometer δ in ppm, J in Hz. MS: *VG AutoSpec-3000* mass spectrometer; in m/z. HR-ESI-MS: *API QSTAR Pulsar-1* mass spectrometer.

2. *Plant Material.* The dried roots of *S. officinalis* were collected in the suburb of Qujing, Yunnan Province of China, in October of 2010, and identified by one of the authors (*J. G. Chen*). A voucher specimen (20101001) was deposited with the Herbarium of the College of Biological Resources and Environment Science, Qujing Normal University, Qujing, Yunnan Province, P. R. China.

3. *Extraction and Isolation.* The roots (5 kg) of *S. officinalis* were cut into small pieces and ground, and then extracted with 80% EtOH (3 × 10 l). After removal of EtOH under reduced pressure, the aq. brownish syrup (2 l) was partitioned successively with petroleum ether (PE), $CHCl_3$, and AcOEt. Concentration of the solvents afford PE (51 g), $CHCl_3$ (81 g), and AcOEt extracts (143 g). The AcOEt-soluble portion was subjected to CC (SiO_2; $CHCl_3$/MeOH from 100:1 to 1:1) to afford *Frs. 1–8*. *Fr. 2* (6.1 g) was submitted to CC (*MCI* gel; MeOH/H_2O from 70% to 95%) to yield three *Subfrs. 2A–2C*. *Subfr. 2B* (1.3 g) was separated by repeated CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1 and MeOH; and SiO_2; $CHCl_3$/MeOH 9:1) to yield **2** (3.7 mg). *Fr. 3* (7.0 g) was divided into four *Subfrs. 3A–3E* by CC (*MCI* gel; MeOH/H_2O from 50% to 95%). *Subfr. 3B* (186 mg) was separated by CC (SiO_2; $CHCl_3$/MeOH from 8:2 to 1:4) and was purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to afford **1** (37.1 mg) and **3** (33.6 mg).

4. *Compounds.* 4.1. *(3β,5α,12β,17β)-17-(2-Hydroxy-1-methylethyl)-4,4,8,10,14-pentamethylgonane-3,12-diol 12-Acetate* (**1**). White amorphous powder. M.p. 172–173°. $[\alpha]_D^{23.3} = +21.56$ ($c = 2.04$, MeOH). IR (KBr): 3535, 2965, 2924, 1740, 1455, 1264, 1035. ^1H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 434 (M^+); HR-ESI-MS (pos.): 457.3290 ($[M + Na]^+$, $C_{27}H_{46}NaO_4^+$; calc. 457.3294).

4.2. *(5α,12β,17β)-12-Hydroxy-17-(2-hydroxy-1-methylethyl)-4,4,8,10,14-pentamethylgon-3-one* (**2**). White amorphous powder. M.p. 190–191°. $[\alpha]_D^{23.3} = +19.26$ ($c = 1.14$, MeOH). IR (KBr): 3438, 2944, 2876, 1715, 1462, 1378, 1033. ^1H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 390 (M^+). HR-ESI-MS (pos.): 413.3035 ($[M + Na]^+$, $C_{25}H_{42}NaO_3^+$; calc. 413.3032).

4.3. *2-[(3β,5α,12β,17β)-3,12-Dihydroxy-4,4,8,10,14-pentamethylgonan-17-yl]propanal* (**3**). White amorphous powder. M.p. 185–186°. $[\alpha]_D^{23.3} = +9.47$ ($c = 1.56$, MeOH). IR (KBr): 3451, 2945, 2806, 1735, 1470, 1376, 1276, 1033. ^1H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 390 (M^+). HR-ESI-MS (pos.): 413.3028 ($[M + Na]^+$, $C_{25}H_{42}NaO_3^+$; calc. 413.3032).

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