New Sesquiterpenoids from *Artemisia anomala*

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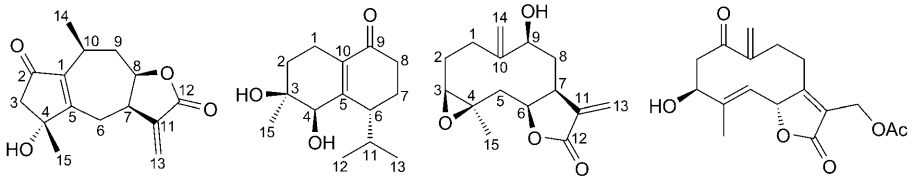
One new guaianolide, anomalactone A (**1**), and one new norcadinane sesquiterpene, anomallenodiol (**2**), along with two germacranolides, anomalactones B and C (**3** and **4**, resp.), were isolated from the aerial part of *Artemisia anomala* S. MOORE. Their structures were determined on the basis of extensive spectroscopic analyses.

Introduction. – *Artemisia anomala* S. MOORE (Chinese name '*Nan-Liu-Ji-Nu*'), a perennial herbaceous plant belonging to the Compositae family, is commonly used in traditional Chinese medicine as an analgesic, antibiotic, and wound-curing agent [1]. Previous phytochemical investigations led to the isolation of flavonoids, coumarins, and sesquiterpene lactones from *A. anomala* [2–7]. Due to the promising bioactivities of this plant, a systematic phytochemical investigation on *A. anomala* has been carried out. Here, we report the isolation and structural elucidation of the four new sesquiterpenoids **1**–**4** from *A. anomala*.



1 **2** **3** **4**

Results and Discussion. – Anomalactone A (**1**) was obtained as a colorless gum, and the molecular formula was established as $C_{15}H_{18}O_4$ (seven degrees of unsaturation) by high-resolution electrospray ion mass spectrometry (HR-ESI-MS; *m/z* 263.1276 ($[M+H]^+$; calc. 263.1278)). The ^{13}C-NMR spectrum (see *Table 2*) exhibited 15 C-atom signals, which were classified by a DEPT experiment into those of two Me, four CH_2, three CH groups (including those of two O-bearing CH groups at δ(C) 75.9 and 78.6), and of six quaternary C-atoms (including a lactone signal at δ(C) 169.6, a C=O signal at δ(C) 204.9, and three olefinic C-atom signals at δ(C) 142.4, 169.5, and 138.3,

respectively, which suggested a sesquiterpenoid skeleton. The ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of the known compound 2α,4α-dihydroxy-7αH,8αH,10αH-guaia-1(5),11(13)-dien-12,8β-olide isolated from *Pulicaria crispa* [8]. The major difference in their NMR spectra was the presence of a C=O group resonance at δ(C) 204.9 in **1** instead of the signal of an oxygenated CH group in the known compound. The C=O group was determined to be C(2)=O by the HMBCs from CH_2(3) to C(2) and from H–C(10) to C(2) (*Fig. 1*). The assignments of all H- and C-atoms for **1** were accomplished by HSQC, ^{1}H,^{1}H-COSY, and HMBC experiments (*Tables 1* and *2*). The above data suggested that the structure of **1** is 2-oxo-4-hydroxyguaia-1(5),11(13)-diene-12,8-olide. The relative configuration of **1** was established by a NOESY experiment, in which the correlations from H–C(7) to H–C(8) and H_α–C(6), from H–C(8) to H_α–C(9) and H–C(10), and from Me(14) to Me(15) and H_β–C(6) indicated that Me(14) and Me(15) are β-oriented, whereas H–C(7) and H–C(8) are α-oriented (*Fig. 2*). Therefore, the structure of compound **1** was established as 4α-hydroxy-2-oxo-7αH,8αH,10αH-guaia-1(5),11(13)-dien-12,8β-olide.

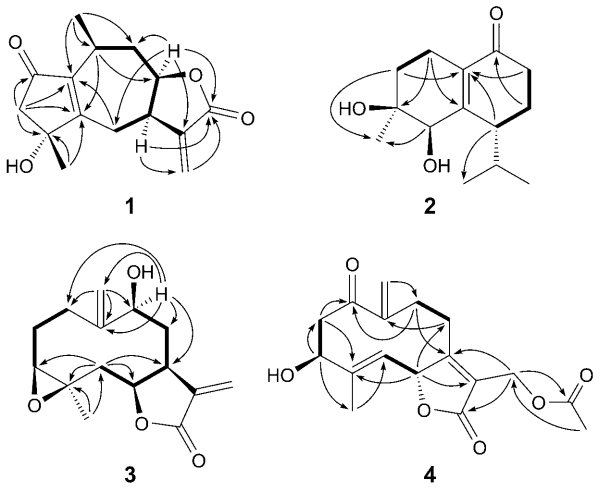


Fig. 1. *Key HMBC* (H → C) *and* ^{1}*H,*^{1}*H-COSY* (—) *correlations of* **1**–**4**

Anomallenodiol (**2**) was obtained as a colorless gum. The molecular formula $C_{14}H_{22}O_3$, with four degrees of unsaturation, was deduced on the basis of HR-ESI-MS (*m*/*z* 239.1637 ([*M* + H]$^+$; calc. 239.1642)). The ^{13}C-NMR (DEPT) data revealed the presence of three Me, four CH_2, and three CH groups, and four quaternary C-atoms, including an α,β-unsaturated CO group and a tetrasubstituted C=C moiety. The ^{1}H-NMR spectrum of **2** exhibited a Me(15) *singlet* at δ(H) 1.22 (*s*), two Me *doublet*s at δ(H) 0.92 (*d*, *J* = 7.0, Me(12)) and 1.06 (*d*, *J* = 7.0, Me(13)), and three CH signals at δ(H) 2.53–2.56 (*m*, H–C(6)), 2.16–2.20 (*m*, H–C(11)), and 3.88 (*s*, H–C(4)). The overall ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of oxyphyllenodiol B [9]. The major differences between the two compounds are the configurations of C(3) and C(4). The relative configuration of **2** was deduced from the NOESY spectrum. The

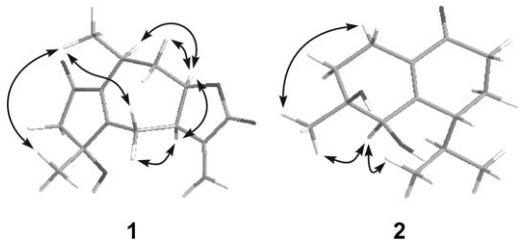
Table 1. *^{1}H-NMR Data* (500 MHz) *of* **1**–**4** (**1**, **3**, and **4** in $CDCl_3$, **2** in CD_3OH). δ in ppm; J in Hz. Assignments were corroborated by ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments.

Position	**1**	**2**	**3**	**4**
1		2.22 (*ddd*, *J* = 1.0, 6.5, 12.5), 2.32 (*dd*, *J* = 6.0, 12.0)	2.43 (*td*, *J* = 2.5, 13.5), 2.55–2.59 (*m*)	
2		1.60 (*dd*, *J* = 2.0, 9.0), 1.68 (*dd*, *J* = 2.0, 9.0)	1.38–1.42 (*m*), 2.15–2.19 (*m*)	3.48 (*dd*, *J* = 8.0, 13.0), 2.70 (*dd*, *J* = 8.0, 13.0)
3	2.57 (*d*, *J* = 10.5)		2.81 (*dd*, *J* = 2.0, 10.5)	4.68 (*dd*, *J* = 8.0, 8.0)
4		3.88 (*s*)		
5			1.44 (*dd*, *J* = 13.0, 13.0), 2.52 (*dd*, *J* = 4.0, 13.0)	4.76 (*d*, *J* = 10.0)
6	2.70 (*dd*, *J* = 1.0, 12.0), 2.80 (*dd*, *J* = 5.0, 12.0)	2.53–2.56 (*m*)	4.79 (*ddd*, *J* = 4.0, 7.0, 13.0)	5.36 (*d*, *J* = 10.0)
7	3.41–3.46 (*m*)	2.00 (*ddd*, *J*= 2.0, 6.0, 12.5)	3.39–3.43 (*m*)	
8	4.65 (*td*, *J* = 4.0, 12.0)	2.30 (*dd*, *J* = 6.0, 10.0), 2.50 (*dd*, *J* = 2.0, 9.0)	1.73 (*ddd*, *J* = 4.5, 8.0, 14.5), 2.10 (*ddd*, *J* = 5.0, 9.0, 14.5)	2.90 (*dd*, *J* = 6.0, 13.5), 2.07–2.10 (*m*)
9	1.90–1.97 (*m*), 2.11–2.15 (*m*)		4.10 (*dd*, *J* = 4.5, 8.0)	2.25 (*dd*, *J* = 5.5, 8.5), 3.05 (*dd*, *J* = 6.5, 12.5)
10	2.79–2.82 (*m*)			
11		2.16–2.20 (*m*)		
12		0.92 (*d*, *J* = 7.0)		
13	6.36 (*d*, *J* = 3.0), 5.71 (*d*, *J* = 3.0)	1.06 (*d*, *J* = 7.0)	6.23 (*d*, *J* = 3.0), 5.56 (*d*, *J* = 3.0)	4.79 (*d*, *J* = 13.0), 4.87 (*d*, *J* = 13.0)
14	1.17 (*d*, *J* = 11.5)		5.07 (*s*), 5.21 (*s*)	5.67 (*d*, *J* = 1.5), 5.76 (*d*, *J* = 0.5)
15	1.50 (*s*)	1.22 (*s*)	1.33 (*s*)	1.78 (br. *s*)
2′				2.11 (*s*)

Table 2. *^{13}C-NMR Data* (125 MHz) *of* **1**–**4** (**1**, **3**, and **4** in $CDCl_3$, **2** in CD_3OH). δ in ppm. Assignments were corroborated by ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments.

Position	**1**	**2**	**3**	**4**
1	142.4	21.3	29.5	199.8
2	204.9	31.1	26.3	46.4
3	50.8	72.0	68.8	74.6
4	75.9	74.3	57.3	141.7
5	169.5	160.4	40.7	123.5
6	24.9	42.7	78.2	79.8
7	41.8	23.7	40.6	168.6
8	78.6	36.2	33.8	24.3
9	35.5	202.4	72.4	32.6
10	28.2	133.7	150.1	148.8
11	138.3	30.8	139.1	123.5
12	169.6	22.1	169.8	172.7
13	123.2	19.7	120.1	55.5
14	20.1	25.1	113.1	124.5
15	26.4		17.7	10.2
1′				170.5
2′				20.7

NOE interactions of Me(15) with Me(13) and Me(12), and of H–C(4) with H_α–C(1) (*Fig. 2*) indicated that Me(15) and H–C(4) are α-oriented, and H–C(6) is β-oriented. Therefore, compound **2** was established as (4*R**,5*R**,6*S**)-5,6-dihydroxy-4-isopropyl-6-methyl-3,4,5,6,7,8-hexahydronaphthalen-1(2*H*)-one.



1 **2**

Fig. 2. *The key NOESY* (H → H) *correlations of* **1** *and* **2**

Anomalactone B (**3**) was obtained as a colorless gum. The molecular formula $C_{15}H_{20}O_4$, with six degrees of unsaturation, was deduced from HR-ESI-MS (*m*/*z* 265.1428 ($[M+H]^+$; calc. 265.1434). The IR spectrum showed strong absorption bands for a C=O group (1759 cm^{-1}) and of a OH group (3449 cm^{-1}). The ^{1}H-NMR (*Table 1*) spectrum exhibited a Me(15) *singlet* at δ(H) 1.33 (*s*), and four olefinic H-atom signals due to two exocyclic C=C bonds at δ(H) 6.23 (*d*, $J = 3.0$) and 5.56 (*d*, $J = 3.0$), 5.07 (*s*) and 5.21 (*s*). The ^{13}C-NMR spectrum (*Table 2*) displayed 15 C-atom signals which were classified by a DEPT experiment into those of one Me, six CH_2, four CH groups (including three O-bearing CH group signals at δ(C) 68.8, 78.2 and 72.4, resp.), and of four quaternary C-atoms (including a lactone signal at δ(C) 169.8, an O-bearing

quaternary C-atom signal at δ(C) 57.3, and two olefinic C-atom signals at δ(C) 139.1 and 150.1). Comparison of the above data with those of deoxyperoxyparthenolide [10] revealed that both of them have the same germacranolide skeleton. The major differences of the two compounds are the positions of a OH and of an epoxy group. The HMBCs of **3** from CH_2(1), H–C(7), H–C(8), and CH_2(14) to C(9) at δ(C) 72.4 suggested that C(9) is substituted by a OH group. The epoxy linkage between C(3) (δ(C) 68.8) and C(4) (δ(C) 57.3) was determined by the relative upfield chemical shift and the HMBCs of CH_2(1), CH_2(5), and Me(15) to C(3), and CH_2(2), H–C(6), and Me(15) to C(4), which were consistent with its molecular formula and the number of degrees of unsaturation (*Fig. 1*). The relative configuration of **3** was determined by the analysis of its NOESY spectrum and the Chem3D molecular modeling studies. The NOESY correlations of H–C(3) with Me(15), H–C(6), H–C(7), and H–C(9) suggested that H–C(3), H–C(6), H–C(7), H–C(9), and Me(15) are on the same side of the molecule. Two possible conformations of **3** were predicted by the computer-generated models (ChemBio3D Ultra 11.0 GAMESS Minimize energy). The coupling constants for **3** between H–C(5) and H–C(6) ($J(5\alpha,6) = 4.0, J(5\beta,6) = 13.0$) requires a dihedral angle of *ca.* 60° between H_α–C(5) and H–C(6), and close to 180° between H_β–C(5) and H–C(6). The structure of **3a** meets these requirements (*Fig. 3*). Thus, compound **3** was identified as 3β,4β-epoxy-9β-hydroxygermacra-10(14),11(13)-dien-12,6β-olide.

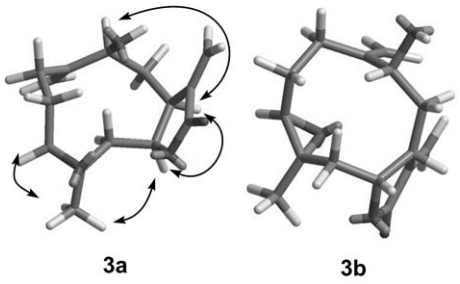


Fig. 3. *Two possible configurations of* **3***, which resulted from molecular modeling.* Key NOESY (H → H) correlations of **3** were indicated.

Anomalactone C (**4**) was obtained as a colorless gum. The molecular formula $C_{17}H_{20}O_6$, with eight degrees of unsaturation, was deduced from HR-ESI-MS (*m*/*z* 343.1157 ($[M + Na]^+$; calc. 343.1152). The ^{1}H-NMR spectrum of **4** was nearly identical with that of 13-bis-*O*-desacetyl-1-oxoafraglaucolide [11], indicating that both of them share a germacranolide skeleton, except an AcO group of **4** located at C(13), which was evident from the presence of chemical-shift values of Me(2′) (δ(H) 2.11), and C(1′) (δ(C) 170.5) and C(2′) (δ(C) 20.7), and the HMBCs of Me(2′) with C(13), and CH_2(13) with C(1′). Thus, compound **4** was established as 3-*O*-desacetyl-1-oxoafraglaucolide. The ^{1}H- and ^{13}C-NMR signals of **4** were fully assigned by means of HSQC and HMBC experiments (*Fig. 1*).

Experimental Part

General. Semi-prep. HPLC: *Waters 600* instrument with *ODS* column (*Agilent Technologies* 250 × 9.4 mm, i.d., 5 μm) and C_{18} guard column with a *2996* photodiode array detector. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, *Qingdao Haiyang Chemical works*) and *ODS* (*Merck*). Optical rotations: *Perkin-Elmer 243B* digital polarimeter. UV Spectra: *TU-1901* spectrometer. IR Spectra: *Nexus-470 FT-IR* (*Nicolet*) spectrometer. NMR Spectra: *Inova 500* spectrometer, operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C; the chemical shifts in δ [ppm] with TMS as internal standard and $CHCl_3$ as solvent. HR-ESI-MS: *Brucker APEX IV FT-MS* spectrometer in positive-ion mode.

Plant Material. The aerial parts of *A. anomala* were collected in Hangzhou Zhejiang, P. R. China in July, 2008. The plant material was authenticated by *P.-F. T.* A voucher specimen was deposited with the Herbarium of the Peking University Modern Research Center for Traditional Chinese Medicine (No. CM20071215).

Extraction and Isolation. Dried aerial parts (300 kg) of *A. anomala* were chopped and extracted three times with 95% EtOH. After evaporation of the solvent under reduced pressure, the residue was suspended in H_2O and extracted with petroleum ether and $CHCl_3$, successively. The residue of the $CHCl_3$ layer (1000 g) was fractionated by SiO_2 CC using a gradient of $CHCl_3$ and MeOH to give 72 fractions. *Frs. 30–35* were subjected to *ODS* open CC (MeOH/H_2O 40:60 to 80:20) to afford subfractions *1–4*. *Subfr. 1* was separated by semi-prep. HPLC (MeOH/H_2O 45:55) to afford **2** (4 mg) and **3** (3 mg), *Subfr. 2* was separated by semi-prep. HPLC (MeOH/H_2O 40:60), and continuously purified by semi-prep. HPLC (MeCN/H_2O 35:65) to yield **4** (7 mg), and *Subfr. 4* was separated by semi-prep. HPLC (MeOH/H_2O 40:60) and then purified by semi-prep. HPLC (MeCN/H_2O 30:70) to afford **1** (4 mg).

Anomalactone A (=*4α-Hydroxy-2-oxo-7α*H,*8α*H,*10α*H-*guaia-1(5),11(13)-dien-12,8β-olide* = *(3a*R*,5*R*,8*S*,9a*R*)-3,3a,4,5,6,8,9,9a-Octahydro-5-hydroxy-5,8-dimethyl-3-methylideneazuleno[6,5-b]furan-2,7-dione*; **1**). Colorless gum. $[\alpha]_D^{22} = -38.0$ ($c = 0.1$, MeOH). IR (KBr): 3432, 2962, 2931, 2873, 1763, 1701, 1647, 1462, 1376, 1335, 1274, 1202, 1169, 1120, 1092, 1057, 997, 957, 853, 813, 755, 668, 630, 594. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 263.1276 ($[M+H]^+$, $C_{15}H_{18}O_4^+$; calc. 263.1278).

Anomallenodiol (=*(4*R*,5*R*,6*S*)-3,4,5,6,7,8-Hexahydro-5,6-Dihydroxy-6-methyl-4-(propan-2-yl)-naphthalen-1(2*H*)-one*; **2**). Colorless gum. $[\alpha]_D^{22} = +18.0$ ($c = 0.1$, MeOH). IR (KBr): 3406, 2958, 2874, 1655, 1377, 1262, 1123, 1023. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 239.1637 ($[M+H]^+$, $C_{14}H_{23}O_3^+$; calc. 239.1642).

Anomalactone B (=*3β,4β-Epoxy-9β-hydroxygermacra-10(14),11(13)-dien-12,6β-olide* = *(1a*S*,5-S*,6a*S*,9a*S*,10a*R*)-Decahydro-5-hydroxy-10a-methyl-4,7-dimethylideneoxireno[8,9]cyclodeca[1,2-b]furan-8(2*H*)-one*; **3**). Colorless gum. $[\alpha]_D^{22} = +59.0$ ($c = 0.1$, MeOH). IR (KBr): 3449, 2964, 2925, 2857, 1759, 1664, 1639, 1447, 1384, 1334, 1261, 1168, 1106, 1068, 1028, 986, 944, 913, 866, 801, 733. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 265.1428 ($[M+H]^+$, $C_{15}H_{21}O_4^+$; calc. 265.1434).

Anomalactone C (=*3*-O-*Desacetyl-1-oxoafraglaucolide* = *[(9*S*,10*E*,11a*R*)-2,4,5,6,7,8,9,11a-Octahydro-9-hydroxy-10-methyl-6-methylidene-2,7-dioxocyclodeca[*b*]furan-3-yl]methyl Acetate*; **4**). Colorless gum. $[\alpha]_D^{22} = +15.0$ ($c = 0.1$, MeOH). IR (KBr): 3482, 2966, 2930, 1755, 1668, 1430, 1369, 1310, 1230, 1178, 1135, 1087, 1027, 989, 916, 732. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 343.1157 ($[M+Na]^+$, $C_{17}H_{20}NaO_6^+$; calc. 343.1152).

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