Two Halogenated Sesquiterpenoids from the Fruits of *Alpinia oxyphylla*

by **Ping Chen**, **Lei Qu**, **Li Tian**, **Pei-Pei Wang**, and **Lan Xiang***

School of Pharmacy, Shandong University, Jinan 250012, P. R. China
(phone: +86-531-88382028; fax: +86-531-88382548; e-mail: xianglan02@sdu.edu.cn)

Two new halogenated eremophilane-type sesquiterpenoids, (11*S*)- and (11*R*)-12-chloronootkaton-11-ol (**1** and **2**, resp.), together with five known sesquiterpenoids, nootkatone (**3**), 7-epiteucrenone B (**4**), oxyphyllenodiol A (**5**), oxyphyllenodiol B (**6**), and alpinenone (**7**), were isolated from the EtOH extract of the fruits of *Alpinia oxyphylla* Miq. The structures were elucidated based on the analyses of their spectroscopic data.

Introduction. – The fruits of *Alpinia oxyphylla* Miq. (Zingiberaceae), '*Yizhiren*' in Chinese, has been used as a traditional Chinese Medicine for the treatment of diarrhea, diuresis, and dementia [1]. In recent years, various types of sesquiterpenoids have been isolated from this plant, and some of them showed inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages or IFN-γ-induced RAW 264.7 murine macrophages [2–6]. In our present study, two new halogenated eremophilane-type sesquiterpenoids, (11*S*)- and (11*R*)-12-chloronootkaton-11-ol (**1** and **2**, resp.), together with five known sesquiterpenoids, nootkatone (**3**) [7], 7-epiteucrenone B (**4**) [8], oxyphyllenodiol A (**5**) [2], oxyphyllenodiol B (**6**) [2], and alpinenone (**7**) [9] (*Fig. 1*), were isolated from the EtOH extract of the fruits of *A. oxyphylla.* Compound **7** was isolated from *A. oxyphylla* for the first time. Herein, we report the isolation and structure elucidation of the new compounds **1** and **2**.

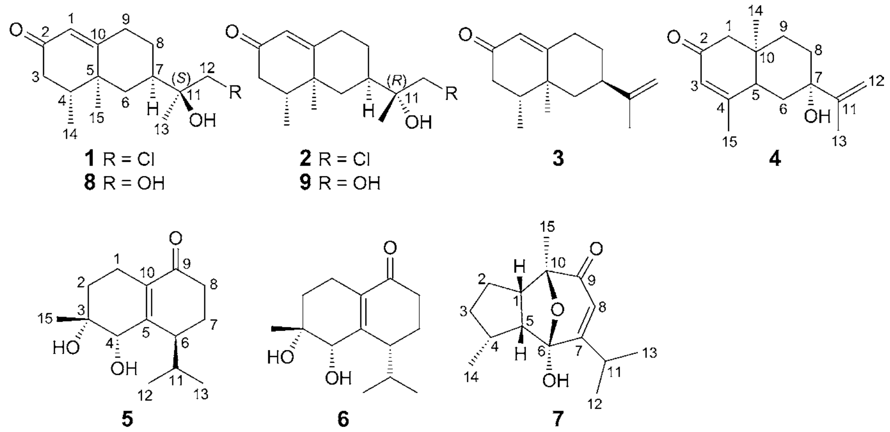

Fig. 1. *Structures of compounds* **1**–**7** *isolated from* A. oxyphylla

Results and Discussion. – Compounds **1** and **2** were separated by prepative HPLC and obtained as colorless oils. They showed yellow or orange color under sunlight, and bright-yellow fluorescence under UV 365 nm when sprayed with 10% H_2SO_4 or 5% vanillin/H_2SO_4. The ESI mass spectra of compounds **1** and **2** exhibited the same characteristic *pseudo*-molecular-ion clusters at 271.3/273.4 (3 : 1, $[M + H]^+$) and 293.3/295.4 (3 : 1, $[M + Na]^+$), indicating that both of them contain one Cl-atom. The molecular formula of **1** was determined as $C_{15}H_{23}ClO_2$, on the basis of HR-ESI-MS signals at *m/z* 271.1464 ($[M + H]^+$) and 293.1281 ($[M + Na]^+$), indicating four degrees of unsaturation. Compound **2** had the same molecular formula according to its HR-ESI-MS signals at *m/z* 271.1463 ($[M + H]^+$) and 293.1279 ($[M + Na]^+$). The ^{1}H-NMR spectrum (*Table 1*) of **1** displayed signals of three Me groups at δ(H) 1.00 (*d*, $J = 4.2$), and at δ(H) 1.14 (*s*) and 1.21 (*s*), of one Cl-bearing CH_2 group at δ(H) 3.65 and 3.70 (2*d*, $J = 10.8$, both 1 H), of one OH group at δ(H) 3.79 (*s*), and of one olefinic H-atom at δ(H) 5.67 (*s*). The ^{1}H-NMR signals of **2** were similar to those of **1**, with slight differences in some chemical shifts (*Table 1*). The ^{13}C-NMR spectrum (*Table 2*) of **1** displayed signals for 15 C-atoms, including those of one CO C-atom at δ(C) 197.6, two olefinic C-atoms at δ(C) 169.8 and 124.0, one O-bearing quaternary C-atom at δ(C) 72.7, one Cl-bearing CH_2 group at δ(C) 53.0, and three Me groups at δ(C) 14.3, 16.3, and 21.3. As shown in *Table 2*, except for the little differences in chemical shifts of C(5), C(6), C(7), and C(8), the remaining ^{13}C-NMR signals of **2** were almost coincident with those of **1**.

Table 1. *^{1}H-NMR Spectral Data of* **1** *and* **2**. At 600 MHz, in $(CD_3)_2CO$; δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1*.

H-Atom	**1**	**2**
H–C(1)	5.67 (br. *s*)	5.67 (br. *s*)
H_α–C(3)	2.07–2.10 (*m*)	2.08–2.10 (*m*)
H_β–C(3)	2.29 (*t*, $J = 15.6$, 15.6)	2.29 (*t*, $J = 15.0$, 16.2)
H_β–C(4)	1.94–2.01 (*m*)	1.97–2.03 (*m*)
H_α–C(6)	2.01–2.05 (*m*)	2.19 (br. *d*, $J = 13.2$)
H_β–C(6)	1.08 (*t*, $J = 13.8$, 12.6)	1.12–1.17 (*m*)
H–C(7)	2.10–2.15 (*m*)	2.10–2.14 (*m*)
H_α–C(8)	2.09–2.13 (*m*)	1.94 (br. *d*, $J = 18.6$)
H_β–C(8)	1.29 (*dd*, $J = 12.6$, 12.6)	1.25–1.30 (*m*)
H_α–C(9)	2.54 (*t*, $J = 15.0$, 13.8)	2.56 (*t*, $J = 14.4$, 14.4)
H_β–C(9)	2.41 (br. *d*, $J = 15.0$)	2.39 (br. *d*, $J = 15.0$)
CH_2(12)	3.65 (*d*, $J = 10.8$), 3.70 (*d*, $J = 10.8$)	3.63 (*d*, $J = 10.8$), 3.68 (*d*, $J = 10.8$)
Me(13)	1.21 (*s*)	1.21 (*s*)
Me(14)	1.00 (*d*, $J = 4.2$)	0.99 (*d*, $J = 6.0$)
Me(15)	1.14 (*s*)	1.13 (*s*)
HO–C(11)	3.79 (*s*)	3.82 (*s*)

Correlations in both of the ^{1}H,^{1}H-COSY spectra of **1** and **2** indicated the presence of two spin systems, including $–CH_2–CH–Me$ and $–CH_2–CH_2–CH–CH_2–$ fragments (*Fig. 2*). Both of the HMBC spectra of **1** and **2** exhibited the correlations from CH_2(3) to C(1), C(2), C(4), C(5), and C(14), from Me(15) to C(10), from CH_2(6) to C(4), C(5), Me(15), C(10), C(7), C(8), and C(11), from Me(13) to C(7), C(11), and C(12),

Table 2. *^{13}C-NMR Spectral Data of (11*S*)- and (11*R*)-Nootkatone-11,12-diol* (**8** and **9**, resp.) [7], *and* **1** *and* **2**. At 150 MHz; δ in ppm. Atom numbering as indicated in *Fig. 1*.

C-Atom	**A**[a])	**B**[a])	**1**[a])	**1**[b])	**2**[b])
H–C(1)	124.4 (*d*)	124.5 (*d*)	124.6 (*d*)	124.0 (*d*)	123.9 (*d*)
C(2)	199.9 (*s*)	199.8 (*s*)	199.6 (*s*)	197.6 (*s*)	197.6 (*s*)
CH_2(3)	42.0 (*t*)	42.0 (*t*)	42.0 (*t*)	41.8 (*t*)	41.8 (*t*)
H–C(4)	40.5 (*d*)	40.6 (*d*)	40.5 (*d*)	40.7 (*d*)	40.7 (*d*)
C(5)	39.6 (*s*)	39.2 (*s*)	39.6 (*s*)	39.5 (*s*)	39.0 (*s*)
CH_2(6)	39.2 (*t*)	38.6 (*t*)	39.2 (*t*)	39.2 (*t*)	38.6 (*t*)
H–C(7)	39.7 (*d*)	39.2 (*d*)	39.9 (*d*)	39.5 (*d*)	39.2 (*d*)
CH_2(8)	26.8 (*t*)	27.9 (*t*)	26.6 (*t*)	26.6 (*t*)	27.4 (*t*)
CH_2(9)	33.0 (*t*)	32.9 (*t*)	32.8 (*t*)	32.6 (*t*)	32.4 (*t*)
C(10)	171.0 (*s*)	170.8 (*s*)	170.1 (*s*)	169.8 (*s*)	169.8 (*s*)
C(11)	74.3 (*s*)	74.2 (*s*)	73.4 (*s*)	72.7 (*s*)	72.6 (*s*)
CH_2(12)	68.2 (*t*)	68.4 (*t*)	53.5 (*t*)	53.0 (*t*)	52.8 (*t*)
Me(13)	20.3 (*q*)	19.9 (*q*)	21.2 (*q*)	21.3 (*q*)	21.4 (*q*)
Me(14)	14.9 (*q*)	15.0 (*q*)	14.9 (*q*)	14.3 (*q*)	14.3 (*q*)
Me(15)	16.9 (*q*)	16.8 (*q*)	16.8 (*q*)	16.3 (*q*)	16.1 (*q*)

[a]) Recorded in $CDCl_3$. [b]) Recorded in $(CD_3)_2CO$.

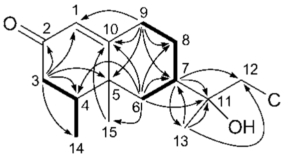

Fig. 2. *^{1}H,^{1}H-COSY correlations* (—) *and key HMBCs* (H → C) *of* **1** *and* **2**

from CH(7) to C(13), C(11), and C(12), and from CH_2(9) to C(1), C(10), C(5), C(8), and C(7). These findings evidenced that **1** and **2** are eremophilane-type sesquiterpenoids.

The relative configurations of **1** and **2** (*Fig. 1*) were determined by NOESY experiments. Compound **1** showed NOE correlations H_β–C(4)/H_β–C(6), H_β–C(3); H_β–C(9)/H_β–C(3), H_β–C(8); H_α–C(6)/Me(14), Me(15); Me(15)/H_α–C(7), H_α–C(8), H_α–C(6); and Me(13)/H_α–C(7), H_α–C(8), and **2** exhibited NOE correlations H_α–C(9)/Me(14), Me(15), H_α–C(7), H_α–C(8); H_α–C(6)/Me(14), Me(15); H_β–C(9)/H–C(1), H_β–C(6), H_β–C(8); and H_β–C(4)/H_β–C(3), indicating that **1** and **2** were halogenated derivatives of nootkatone (**3**). The absolute configuration at C(11) was determined through comparison with the structures of the reported eremophilane-type sesquiterpenoids, (11*S*)- and (11*R*)-nootkatone-11,12-diol (**8** and **9**, resp.), which were produced from biotransformation of (+)-nootkatone by *Aspergillus niger* [7]. As shown in *Table 2*, the C-atom signals of **1** and **2** were very similar to those of **8** and **9**, except for the signal of C(12), which was substituted by a Cl-atom in **1** and **2**. As reported, the absolute configurations of **8** and **9** could not be characterized by CD spectra, because both thiocarbonates of **8** and **9** showed negative *Cotton* effects at *ca.* 310 nm in CD spectra. The (*S*)-configuration at C(11) of **8** was finally established based on X-ray crystallographic analysis of the thiocarbonate of **8** (*Fig. 3*) [7]. Interestingly,

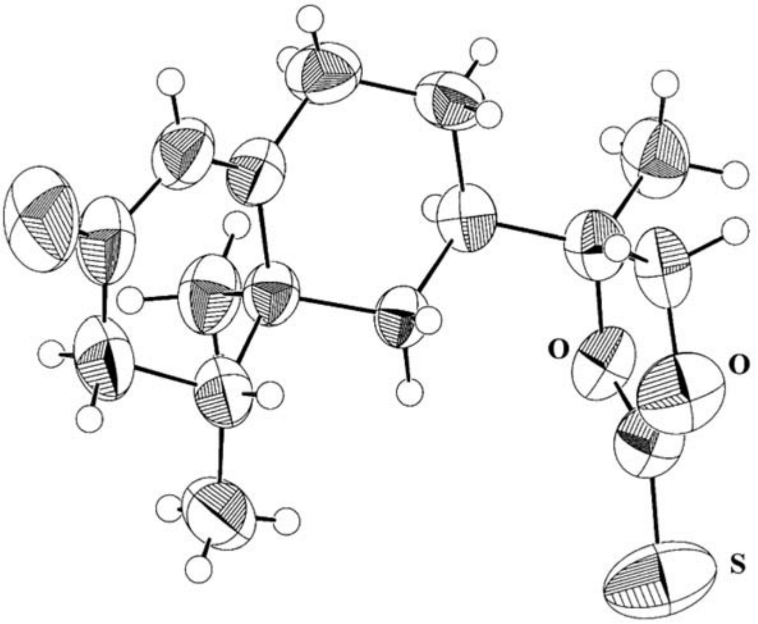

Fig. 3. *ORTEP Drawing of the thiocarbonate of* **8** (from [7])

some regularities can be deduced through comparison of the C-atom signals of **8** and **9**, which may be helpful for the determination of the relative configuration at C(11): the signals of C(5), C(6), and C(7) of **8** appeared at a lower field (+0.4, +0.6, +0.5 ppm, resp.), and the signal of C(8) appeared at a higher field (−1.1 ppm), compared to those of **9** (*Table 2*). Similarly, the signals at C(5), C(6), and C(7) of **1** appeared at a lower field (+0.5, +0.6, +0.3 ppm, resp.), and the signal of C(8) appeared at a higher field (−0.8 ppm) compared to those of **2**, demonstrating that **1** and **2** were (11*S*)- and (11*R*)-12-chloronootkatone-11-ol, respectively.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Haiyang Chemical Group Co. Ltd.*, P. R. China), *ODS-C18* (75 μm, *YMC Co.*, Japan), *MCI* gel (*CHP-20P*, 75–150 μ, *Mitsubishi Chemical Co.*, Japan), and *Sephadex LH-20* (*Pharmacia Fine Chemicals*, USA). Semi-prep. HPLC: *Shimadzu Prominence LC-20A* liquid chromatography, with *LC-20AT* pumps, *SPD-20A* UV detector (*Shimadzu Co.*, Japan), and a *YMC-Pack ODS-A* column (250 mm × 10 mm, 5 μm) (*YMC Co.*, Japan). TLC: silica-gel *GF254* plates (*Qingdao Haiyang Chemical Group Co. Ltd.*, P. R. China); visualization by heating the plates sprayed with 10% H_2SO_4 or 5% vanillin/H_2SO_4 reagent. UV Spectra: *UV-2450* spectrophotometer (*Shimadzu Co.*, Japan). Optical rotations: *Gyromat-Hp* digital automatic polarimeter (*Kernchen Co.*, Germany). CD Spectra: *Chirascan* spectrophotometer (*Applied Photophysics Ltd.*, UK). NMR Spectra: *Bruker AV-600*, δ in ppm, with TMS as an internal standard, *J* in Hz. ESI-MS Spectra: *API 4000* mass spectrometer (*Applied Biosystems Co.*, USA). HR-ESI-MS: *LTQ-Orbitrap* mass spectrometer (*Thermo Fisher Co.*, USA), in *m*/*z*.

Plant Material. The fruits of *Alpinia oxyphylla* MIQ. were purchased from Shuyupingmin Pharmacy (Jinan, P. R. China) in March 2011 and authenticated by *L. X.*, School of Pharmacy, Shandong

University, P. R. China. A voucher specimen (No. 090801) has been deposited with the Laboratory of Pharmacognosy, School of Pharmacy, Shandong University.

Extraction and Isolation. The dried fruits of *A. oxyphylla* (5 kg) were powdered and extracted with 95% EtOH (4 × 25 l) at r.t. The combined extracts were evaporated to yield a crude extract (450 g), which was then suspended in 20% EtOH and partitioned with petroleum ether (PE), AcOEt, and BuOH successively. The AcOEt extract (210 g) was separated by CC (SiO_2, PE/AcOEt 20 : 1 to 1 : 1) to yield twelve fractions, *Frs. 1–12*. *Fr. 3* (30.5 g) was subjected to CC (*Sephadex LH-20*; EtOH) to yield three fractions *Frs. 3.1–3.3*. *Fr. 3.2* (5.2 g) was purified by CC (1. SiO_2, PE/CH_2Cl_2 1 : 9; 2. *ODS-C18*, EtOH/H_2O 1 : 1 to 4 : 1) to afford compound **3** (700 mg). *Fr. 5* (1.6 g) was subjected to CC (*MCI*, EtOH/H_2O 35 : 65 to 70 : 30) to afford eight subfractions, *Frs. 5.1–5.8*. Compound **7** (5 mg) was obtained from recrystallisation of *Fr. 5.3*. *Fr. 8* (11.1 g) was subjected to CC (*ODS-C18*, EtOH/H_2O 2 : 8 to 7 : 3) to yield nine subfractions, *Frs. 8.1–8.9*. *Fr. 8.2* (1.2 g) was further purified by CC (1. *MCI*, EtOH/H_2O 24 : 76 to 7 : 3; 2. SiO_2, CH_2Cl_2/AcOEt 20 : 1 to 4 : 1) to yield **4** (28.1 mg). *Fr. 8.3* (750 mg) was purified by CC (SiO_2, CH_2Cl_2/AcOEt 20 : 1 to 5 : 1) and semi-prep. HPLC (MeOH/H_2O 6 : 4) to yield **1** (9.5 mg) and **2** (4.7 mg). *Fr. 9* (25 g) was separated by CC (SiO_2, CH_2Cl_2/AcOEt 100 : 1 to 1 : 1) to afford ten subfractions, *Frs. 9.1–9.10*. *Fr. 9.4* (5.1 g) was purified by repeated CC (SiO_2, CH_2Cl_2/AcOEt) to furnish twelve subfractions, *Frs. 9.4.1–9.4.12*. Compound **5** (72.6 mg) was obtained from recrystallization of *Fr. 9.4.1*. Compound **6** (79.3 mg) was obtained from *Fr. 9.4.2* by semi-prep. HPLC (MeOH/H_2O 75 : 25).

(11S)-12-Chloronootkaton-11-ol (= *(4R,4aS,6R)-6-[(2S)-1-Chloro-2-hydroxypropan-2-yl]-4,4a,5,6,7,8-hexahydro-4,4a-dimethylnaphthalen-2(3H)-one*; **1**). Colorless oil. $[\alpha]_D^{20} = +41.0$ ($c = 0.1$, MeOH). UV (MeOH): 238 (4.72). CD ($c = 0.1$, MeOH): 317 (-0.66). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 271.1464 ($[M+H]^+$, $C_{15}H_{24}{}^{35}ClO_2^+$; calc. 271.1465), 293.1281 ($[M+Na]^+$, $C_{15}H_{23}{}^{35}ClNaO_2^+$; calc. 293.1284).

(11R)-12-Chloronootkaton-11-ol (= *(4R,4aS,6R)-6-[(2R)-1-Chloro-2-hydroxypropan-2-yl]-4,4a,5,6,7,8-hexahydro-4,4a-dimethylnaphthalen-2(3H)-one*; **2**). Colorless oil. $[\alpha]_D^{20} = +6.0$ ($c = 0.1$, MeOH). UV (MeOH): 238 (3.93). CD ($c = 0.1$, MeOH): 312 (-0.19). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 271.1463 ($[M+H]^+$, $C_{15}H_{24}{}^{35}ClO_2^+$; calc. 271.1465), 293.1279 ($[M+Na]^+$, $C_{15}H_{23}{}^{35}ClNaO_2^+$; calc. 293.1284).

The crystal data for the thiocarbonate of (11S)-nootkatone-11,12-diol (**8**; from [7]): monoclinic, space group $P2_1$, $a = 8.710$ (0) Å, $b = 8.448$ (0) Å, $c = 10.988$ (0) Å, $\beta = 102.428$, $V = 789.599976$ (0) Å^3, $Z = 2$, $D_x = 1.530$ Mg/m^3, $D_m = 1.500$ Mg/m^3, $\mu(\mathrm{Mo}K_\alpha) = 2.09$ mm^{-1}, Eta; +1.9. Final R and R_w were 0.044 and 0.074, resp., for 1186 reflections.

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