Three Unusual New Sesquiterpenes from *Alpinia oxyphylla*

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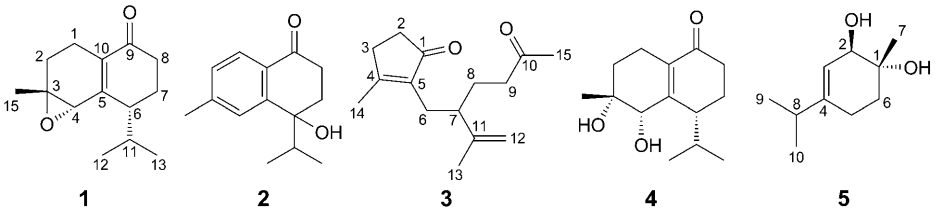
Two new norcadinene sesquiterpenes oxyphyllones C and D (**1** and **2**, resp.), and one new 1,10-secoguaiane sesquiterpene, (+)-mandassidion (**3**), together with two known compounds, oxyphyllenodiol B (**4**) and (1*R*,2*R*)-*p*-menth-3-ene-1,2-diol (**5**), were isolated from the fruits of *Alpinia oxyphylla*. Their structures were determined on the basis of spectroscopic analysis, including 2D-NMR spectroscopic techniques. Compounds **1**, **2**, and **3** exhibited no cytotoxicity against three cancer cell lines.

Introduction. – The plant of *Alpinia oxyphylla* MIQ. (Zingibereae) is widely distributed in South China and used as a folk medicine to treat intestinal disorders, urosis, diuresis, ulceration, and dementia [1–3]. Previous phytochemical investigations of this plant have led to the isolation of bioactive compounds including many sesquiterpenes [1][2]. In our present research, three unusual new sesquiterpenes including two 14-norcadinene sesquiterpenes oxyphyllones C (**1**) and D (**2**), and a 1,10-secoguaiane sesquiterpene, (+)-mandassidion (**3**), together with two known compounds, oxyphyllenodiol B (**4**) [1] and (1*R*,2*R*)-*p*-menth-3-ene-1,2-diol (**5**) [4][5], were isolated from the fruits of *A. oxyphylla*. Compounds **1**, **2**, and **3** were tested for their activities towards human-tumor A549, HT-29, and SGC-7901 cell lines, none of them showed cytotoxicities in these assays at 10 μg/ml according to the method described in [6]. In this article, we mainly report the isolation and the structure elucidation of the new sesquiterpenes.



Results and Discussion. – Oxyphyllone C (**1**) was obtained as a colorless oil. The molecular formula $C_{14}H_{20}O_2$ with five degrees of unsaturation was deduced on the basis of HR-ESI-MS (*m*/*z* 243.1357 ([*M* + Na]$^+$; calc. 243.1360)). The ^{1}H-NMR data (*Table*) showed three Me signals at δ(H) 0.99 (*d*, *J* = 6.8, Me(12)), 0.91 (*d*, *J* = 6.8, Me(13)), and 1.48 (*s*, Me(15)), three CH signals at δ(H) 3.18 (*s*, H–C(4)), 2.30–2.36 (*m*, H–C(6))

and 1.99–2.15 (*m*, H–C(11)), and four CH_2 signals. The ^{13}C-NMR and DEPT spectra revealed the presence of an α,β-conjugated ketone system, resonating at δ(C) 197.5 (*s*, C(9)), 155.2 (*s*, C(5)), and 133.9 (*s*, C(10)), respectively, and an epoxy linkage between C(3) (δ(C) 63.3 (*s*)) and C(4) (δ(C) 57.9 (*d*)). The above information suggested that compound **1** was an unusual degraded cadinene sesquiterpene, similar to the known compound oxyphyllenodiol B [1].

Table. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2** *at 400/100 MHz, Respectively.* In $CDCl_3$; δ in ppm, *J* in Hz.

	1		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	2.65 (br. *dd*, *J* = 5.9, 16.9, H_α), 1.82–1.96 (*m*, H_β)	18.1 (*t*)	7.88 (*d*, *J* = 8.0)	127.4 (*d*)
2	1.50–1.55 (*m*, H_α), 1.99–2.15 (*m*, H_β)	26.8 (*t*)	7.19 (*d*, *J* = 8.0)	128.8 (*d*)
3		63.3 (*s*)		144.1 (*s*)
4	3.18 (*s*)	57.9 (*d*)	7.43 (br. *s*)	126.2 (*d*)
5		155.2 (*s*)		148.7 (*s*)
6	2.30–2.36 (*m*)	44.8 (*d*)		73.7 (*s*)
7	1.82–1.96 (*m*, H_α), 1.99–2.15 (*m*, H_β)	23.3 (*t*)	2.04–2.16 (*m*), 2.36–2.42 (*m*)	33.0 (*t*)
8	2.45–2.52 (*m*, H_α), 2.30–2.36 (*m*, H_β)	34.5 (*t*)	2.63–2.79 (*m*)	34.6 (*t*)
9		197.5 (*s*)		197.4 (*s*)
10		133.9 (*s*)		128.8 (*s*)
11	1.99–2.15 (*m*)	29.9 (*d*)	2.04–2.16 (*m*)	34.9 (*d*)
12	0.99 (*d*, *J* = 6.8)	21.3 (*q*)	1.01 (*d*, *J* = 6.8)	17.5 (*q*)
13	0.91 (*d*, *J* = 6.8)	19.5 (*q*)	0.93 (*d*, *J* = 6.8)	16.3 (*q*)
15	1.48 (*s*)	21.5 (*q*)	2.43 (*s*)	44.0 (*q*)

The planar structure of **1** was determined by ^{1}H,^{1}H-COSY and HMBC correlations (*Fig. 1*). In the ^{1}H,^{1}H-COSY spectrum, two spin systems corresponding to CH_2(1)/CH_2(2) and to Me(12), and Me(13)/H–C(11)/H–C(6)/CH_2(7)/CH_2(8) were observed, which were extended by HMBC correlations of CH_2(1) with C(9) and C(10), of Me(15) with C(2), C(3), and C(4), of H–C(4) with C(5), C(6) and C(10), of H–C(6) with C(5) and C(10), and CH_2(8) with C(9).

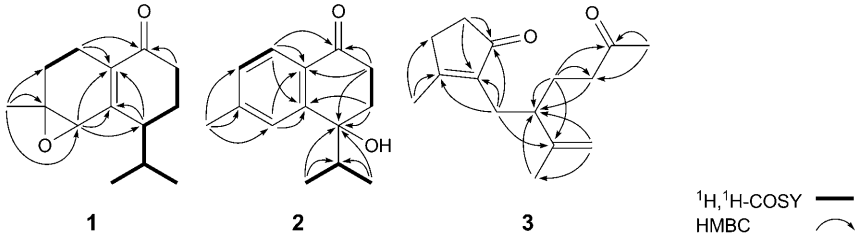
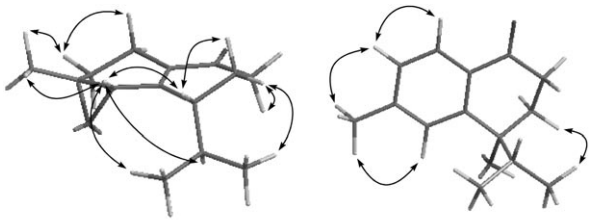


Fig. 1. *^{1}H,^{1}H-COSY and key HMBC for compounds* **1**–**3**

From the key NOESY correlations (*Fig. 2*) between H–C(4) and Me(12) (δ(H) 0.99) and H_β–C(6) (δ(H) 2.30–2.36), and by comparing with the data of oxyphyllenodiol B, the Me–C(3) and iPr–C(6) groups should be in β- and α-orientation,

respectively [1], which was further supported by the computer-generated (Gaussian 03, B3LYP/6-31G**) interatomic distance of H–C(4) to H–C(6), 2.41 Å < 3 Å [7][8]. However, when the Me–C(3) and iPr–C(6) groups were fixed in α-orientation, the calculated interatomic distance of H–C(4) and H–C(6) was 3.53 Å > 3 Å. Moreover, from the biosynthetic point of view, the co-occurrence of compounds **1** and oxyphyllenodiol B in the same plant suggested that oxyphyllenodiol B could be the precursor of **1**, which was in agreement with the above deduction. Thus, the relative configuration of **1** was established.



1 **2**

Fig. 2. *Key ROESY correlations for* **1** *and* **2**

Oxyphyllone D (**2**) was obtained as a colorless, optically inactive oil. Its HR-ESI-MS (*m/z* 241.1206 ($[M+Na]^+$)) showed the molecular formula $C_{14}H_{18}O_2$. ^{13}C-NMR and DEPT spectra (*Table*) indicated 14 C-atom signals due to seven sp^2 and seven sp^3 C-atoms (5 C, 4 CH, 2 CH_2, 3 Me). Moreover, the seven sp^2 C-atom signals were ascribed to a CO and a 1,3,4-trisubstituted aromatic ring, which was further confirmed by the ^{1}H-NMR spectrum signals at δ(H) 7.88 (*d*, *J* = 8.0, 1 H), 7.19 (*d*, *J* = 8.0, 1 H), and 7.43 (br. *s*, 1 H). According to the ^{1}H,^{1}H-COSY, key HMBC and NOESY correlations, a norcadinene-type skeleton of **2** was established (*Figs. 1* and *2*), with the OH group located at C(6), based on the HMBC correlations: the cross-peaks from Me(12) and Me(13) to C(6), from CH_2(7) and CH_2(8) to C(6) and with a C(9)=O group, derived from the HMBC correlations: the cross-peaks from H–C(1) and CH_2(8) to C(9). Since this compound which is similar to 4-hydroxy-4,7-dimethyl-1-tetralone [9][10], showed no optical activity, it must be a racemic mixture.

(+)-Mandassidion (**3**; ($[\alpha]_D^{28} = +33.22$) was obtained as a colorless oil. The molecular formula of **3** was revealed as $C_{15}H_{22}O_2$ by HR-ESI-MS (*m/z* 257.1517 ($[M+Na]^+$)), ^{13}C-NMR, and DEPT spectra. The NMR data as compared with literature suggested that compound **3** was a 1,10-secoguaiane sesquiterpene with the same planar structure to mandassidion ($[\alpha]_D = -9.1$) [11], which was supported by HSQC and key HMBC correlations (*Fig. 1*). Comparison of the optical value of mandassidion, compound **3** was determined as an enantiomer of mandassidion.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 100–200 or 200–300 mesh), and silica gel *H* (60 μm), both obtained from *Qingdao Marine Chemical Co.*, P. R. China. TLC: silica gel *GF254*. Semiprep. reversed-phase (RP) HPLC: *Agilent 1100* liquid chromatograph, with a *Zorbax SB-C_{18}* column. MCI: *CHP-20P*. *Sephadex*: *LH-20*. Optical rotations: *Jasco DIP-370* digital polarimeter. UV

Spectra: *Shimadzu 210A* double-beam spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrometer; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker DRX-500* and *Bruker AM-400* instruments, with Me_4Si as internal standard; δ in ppm. EI-MS: *VG Auto Spec-3000* mass spectrometer; in *m/z* (rel. %). ESI and HR-ESI-MS: *API Qstar Pulsar* instrument.

Plant Material. The fruits of *Alpinia oxyphylla* were bought from Kunming medicinal market, Kunming, Yunnan Province, P. R. China and identified by Prof. *Ning-Hua Tan*.

Extraction and Isolation. The dried and powdered fruits of *Alpinia oxyphylla* (15 kg) were extracted with acetone/H_2O (70%) under reflux for 8 h (5×30 l). The resulting residue was partitioned between petroleum ether (PE) and H_2O, AcOEt and H_2O, and then BuOH and H_2O. The AcOEt extract (480 g) was separated by CC on SiO_2, eluting with PE/acetone (9:1 to 1:1) to yield ten fractions (*Frs. 1–10*). *Fr. 1* (16 g) was subjected to CC (SiO_2, PE/$CHCl_3$/acetone 3:1:0.1) to afford two subfractions (*Fr. 1.1* and *Fr. 1.2*). *Fr. 1.2* was further purified by repeated CC (*RP-18*) and HPLC (MeOH/H_2O 6:4) to yield **1** (3 mg). *Fr. 4* (49 g) was subjected to CC (SiO_2, $CHCl_3$/AcOEt, 100:1 to 9:1) to afford four subfractions (*Frs. 4.1–4.4*). *Fr. 4.2* was further purified by repeated CC (*MCI*) and *Sephadex* (*LH-20*) and HPLC (MeCN/H_2O 3:7) to yield **2** (3 mg) and **3** (24 mg). *Fr. 8* (5 g) was subjected to CC (*RP-18*, MeOH/H_2O 85:15 to 1:1) to afford three subfractions (*Frs. 8.1–8.3*). *Fr. 8.3* was further purified by CC (SiO_2, $CHCl_3$/Me_2CO 3:1 and PE/AcOEt 1:2) to yield **4** (10 mg) and **5** (10 mg).

Oxyphyllone C (=*(1a*R*,7*R*,7b*S*)-1a,3,5,6,7,7b-Hexahydro-1a-methyl-7-(propan-2-yl)naphtho[1,2-*b*]oxiren-4(2*H*)-one*; **1**). Colorless oil. $[\alpha]_D^{23} = -308.19$ ($c = 0.15$, $CHCl_3$). UV (MeOH): 196 (3.53), 262 (3.94). IR (KBr): 3425, 2958, 2925, 1662. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 243.1357 ($[M+Na]^+$, $C_{14}H_{20}NaO_2^+$; calc. 243.1360).

Oxyphyllone D (=*3,4-Dihydro-4-hydroxy-6-methyl-4-(propan-2-yl)-naphthalen-1(2*H*)-one*; **2**). Colorless oil. $[\alpha]_D^{27} = 0$ ($c = 0.35$, $CHCl_3$). UV (MeOH): 209 (4.19), 260 (4.00). IR (KBr): 3442, 2966, 1670, 1605. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 241.1206 ($[M+Na]^+$, $C_{14}H_{18}NaO_2^+$; calc. 241.1204).

(+)-*Mandassidion* (=*3-Methyl-2-[5-oxo-2-(prop-1-en-2-yl)hexyl]cyclopent-2-en-1-one*; **3**). Colorless oil. $[\alpha]_D^{28} = +33.22$ ($c = 0.15$, $CHCl_3$). UV (MeOH): 236 (3.72). ^{1}H-NMR ($CDCl_3$, 400 MHz): 4.67 (br. *s*, H_a–C(12)); 4.57 (br. *s*, H_b–C(12)); 2.45–2.48 (*m*, CH_2(2)); 2.30–2.40 (*m*, CH_2(3), CH_2(9)); 2.17–2.28 (*m*, H–C(7), CH_2(6)); 2.08 (*s*, Me(15)); 2.00 (*s*, Me(14)); 1.49–1.62 (*m*, CH_2(8)); 1.59 (*s*, Me(13)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 209.5 (*s*, C(10)); 209.0 (*s*, C(1)); 171.0 (*s*, C(4)); 146.3 (*s*, C(11)); 138.8 (*s*, C(5)); 112.4 (*t*, C(12)); 45.1 (*d*, C(7)); 41.5 (*t*, C(9)); 34.2 (*t*, C(3)); 31.5 (*t*, C(2)); 30.0 (*q*, C(15)); 27.6 (*t*, C(6)); 26.2 (*t*, C(8)); 18.0 (*q*, C(13)); 17.5 (*q*, C(14)). HR-ESI-MS: 257.1517 ($[M+Na]^+$, $C_{15}H_{22}NaO_2^+$; calc. 257.1515).

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