Secoprezizaane Sesquiterpene Lactones from *Illicium micranthum*

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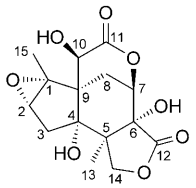
Three new secoprezizaane sesquiterpene lactones, (1*R*,2*S*)-1,2-epoxyneomajucin (**1**), (2*R*)-2-hydroxyneomajucin (**2**), and (2*R*)-2-hydroxymajucin (**3**), along with six known compounds (**4**–**9**), were isolated from the poisonous shrub *Illicium micranthum*. Their structures were established by in-depth analyses of spectroscopic and mass-spectrometric data.

Introduction. – The plants of the genus *Illicium* are mainly distributed in East Asia and in the southeast of North America. In southern China, there are more than 25 widely distributed *Illicium* species known [1]. Previous phytochemical investigations of this genus has led to the isolation of many sesquiterpene lactones endowed with a unique secoprezizaane C-skeleton [2]. Some of them were shown to exhibit neurotoxic [3] or neurotrophic [4] activities.

Illicium micranthum, a poisonous shrub used as a traditional pesticide [1], has not been investigated so far in terms of chemical constituents. Herein, we describe the isolation and structure elucidation of three new sesquiterpene lactones (**1**–**3**) from *I. micranthum*, which were obtained together with the known sesquiterpene lactones pseudomajucin (**4**) [5], neomajucin (**5**) [6], majucin (**6**) [6], (2*S*)-2-hydroxyneomajucin (**7**) [6], 3,4-dehydro-2-oxoneomajucin (**8**) [6], and 2-oxoneomajucin (**9**) [6].

Results and Discussion. – Compound **1** was assigned the molecular formula $C_{15}H_{18}O_8$, as determined by HR-ESI-MS (*m*/*z* 349.0902 ([*M*+Na]$^+$; calc. 349.0899)), corresponding to seven degrees of unsaturation. Its IR spectrum displayed absorptions due to OH groups (3514, 3445, 3279), a γ-lactone (1793), and a δ-lactone (1737 cm^{-1}). The ^{13}C-NMR spectrum of **1** exhibited 15 signals, including two characteristic lactone C=O resonances at δ(C) 176.8 and 173.1. Detailed comparison of the ^{1}H- and ^{13}C-NMR data of **1** (*Tables 1* and *2*, resp.) with those of neomajucin (**5**) [6] indicated a close structural analogy, except for C(1) and C(2). Compared to **5**, the NMR spectrum of **1** suggested an *oxygenated* methine (H–C(1)) and an *oxygenated* quaternary C-atom (C(1)) instead of a non-oxygenated CH and a CH_2 group, respectively. Moreover, the molecular formula of **1** exhibited one degree of unsaturation more than that of neomajucin (**5**). From this evidence, an additional epoxide ring [7] between C(1) and C(2) was inferred in the case of **1**.



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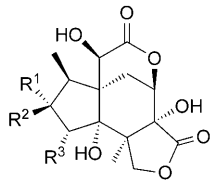
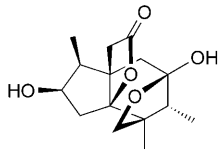


	R^1	R^2	R^3
2	H	OH	H
3	OH	H	OH
5	H	H	H
6	H	H	OH
7	OH	H	H
9	=O		H



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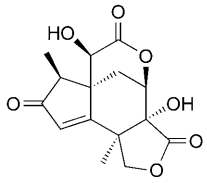

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Table 1. *^{1}H-NMR Data of* **1**–**3**. At 400 MHz in C_5D_5N; δ in ppm, J in Hz.

Atom[1])	**1**	**2**	**3**
H_α–C(1)	–	3.11–3.15 (*m*)	3.06–3.11 (*m*)
H_α–C(2)	–	4.70–4.81 (*m*)	–
H_β–C(2)	3.83 (*s*)	–	4.57 (*t*, *J*=7.5)
H_α–C(3)	2.04 (*d*, *J*=13.6)	2.74 (*dd*, *J*=13.6, 7.8)	4.91 (*d*, *J*=7.9)
H_β–C(3)	2.34 (*d*, *J*=13.6)	2.14 (*dd*, *J*=13.6, 4.4)	–
H_α–C(7)	5.11 (*s*)	5.12 (*d*, *J*=2.4)	5.09–5.15 (*m*)
H_α–C(8)	3.21 (*d*, *J*=4.0)	2.07 (*dd*, *J*=14.3, 2.8)	3.17 (*d*, *J*=14.0)
H_β–C(8)	2.22 (*dd*, *J*=14.0, 2.4)	3.05 (*dd*, *J*=14.3, 2.1)	2.09 (*dd*, *J*=14.0, 2.9)
H_α–C(10)	4.92 (*d*, *J*=4.4)	4.67 (*d*, *J*=3.1)	4.63 (*d*, *J*=4.6)
Me(13)	1.55 (*s*)	1.66 (*s*)	1.71 (*s*)
H_α–C(14)	4.07 (*d*, *J*=11.0)	4.18 (*d*, *J*=11.0)	4.34 (*d*, *J*=11.0)
H_β–C(14)	5.00 (*d*, *J*=11.0)	4.94 (*d*, *J*=11.0)	5.07 (*d*, *J*=11.0)
Me(15)	1.58 (*s*)	1.99 (*d*, *J*=7.4)	1.33 (*d*, *J*=7.0)
10-OH	9.33 (br. *d*, *J*=4.4)	9.60 (br. *d*, *J*=3.1))	9.13 (br. *d*, *J*=4.6)

The proposed structure of **1** was corroborated by HMBC correlations between Me(15) and C(1) and C(2), and between CH_2(3) and C(1) and C(2) (*Fig. 1*). In a ROESY experiment (*Fig. 2*), Me(15) showed a cross-peak with H_β–C(2), which indicated that the epoxide ring was α-oriented. From these data, the structure of compound **1** was assigned as (1*R*,2*S*)-1,2-epoxyneomajucin[1]).

Compound **2** was assigned the molecular formula $C_{15}H_{20}O_8$, as educed from its HR-ESI-MS data (*m*/*z* 351.1050 ($[M+Na]^+$; calc. 351.1055). Its IR spectrum showed the presence of OH groups (3581, 3537, 3477, 3320), a γ-lactone ring (1779), and a δ-lactone ring (1733 cm^{-1}). The NMR data of **2** (*Tables 1* and *2*) also indicated a secoprezizaane skeleton [6]. The only difference between **2** and neomajucin (**5**) was that a CH_2 signal in the latter was replaced by an oxygenated CH in **2**.

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[1]) Arbitrary C-atom numbering. For systematic names, see the *Exper. Part*.

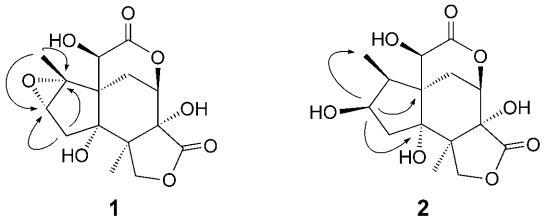


Fig. 1. *Key HMBC correlations for compounds* **1** *and* **2**

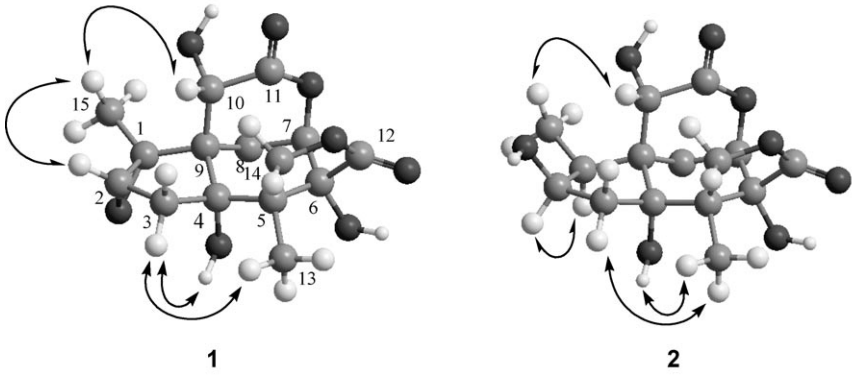


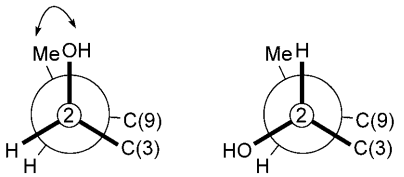
Fig. 2. *Selected REOSY correlations for compounds* **1** *and* **2**[1])

Table 2. *^{13}C-NMR Data of* **1**–**3**. At 100 MHz in C_5D_5N; δ in ppm.

Position[1])	**1**	**2**	**3**	**5**	**6**
1	66.8	43.2	48.2	39.4	38.0
2	64.2	72.2	76.8	31.4	42.9
3	34.9	43.3	71.7	31.6	72.7
4	80.5	81.8	82.5	84.1	82.8
5	47.4	47.2	47.4	47.5	47.5
6	80.0	79.9	79.9	79.6	79.9
7	80.8	80.5	80.6	80.5	80.6
8	24.3	27.5	26.9	27.5	27.1
9	52.3	51.3	49.2	51.0	51.5
10	70.6	70.1	69.7	70.7	70.3
11	173.1	173.8	174.8	174.8	174.7
12	176.8	177.7	178.0	177.2	177.6
13	21.8	12.5	21.1	21.4	20.9
14	71.8	72.4	72.5	72.6	72.4
15	14.2	9.0	12.7	14.3	14.1

In the HMBC spectrum of **2** (*Fig. 1*), H_α–C(2) (δ(H) 4.70–4.81) exhibited correlations with C(4), C(9), and C(15) at δ (C) 81.8, 51.3, and 9.0, respectively, indicating a 2-OH group. The signal at δ(H) 4.70–4.81, as well as the ^{1}H,^{1}H-COSY correlations of Me(15)/H_α–C(1)/H_α–C(2)/CH_2(3), further confirmed the above deduction. Compared

to the known (2*S*)-2-hydroxyneomajucin (**7**), compound **2** demonstrated an upfield ^{1}H-NMR chemical shift for Me(15) due to the γ-gauche effect (*Fig. 3*) [8]; this, in turn, implied the presence of a 2β-OH group. In a ROESY experiment (*Fig. 2*), H_α–C(1) at δ(H) 3.11–3.15 showed a correlation with H_α–C(2) at δ(H) 4.70–4.81, which also supported the β-orientation of the OH-group at C(2). Thus, the structure of **2** was identified as (2*R*)-2-hydroxyneomajucin.



2 **7**

Fig. 3. Gauche *effect for compound* **2** *and its epimer* **7** *illustrated by* Newman *projections along C(2)–C(1)*

The molecular formula of compound **3** was established as $C_{15}H_{20}O_9$, based on the analysis of EI-MS and ^{13}C-NMR data, and confirmed by HR-ESI-MS (*m*/*z* 367.1013 ([*M*+Na]$^+$; calc. 367.1005)). The IR spectrum of **3** showed absorption bands for OH groups (3552, 3509, 3451, 3209), a γ-lactone (1783), and a δ-lactone (1731 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **3** (*Tables 1* and *2*, resp.) also indicated a secoprezizaane sesquiterpene [6]. Two characteristic lactone C=O signals at δ(C) 178.0 and 174.8 were observed in the ^{13}C-NMR spectrum. In addition, the ^{1}H- and ^{13}C-NMR data of **3** revealed great similarities with those of majucin (**6**), except for one more OH group at C(2) in **3**, which was supported by HMBC correlations between H_β–C(2) at δ(H) 4.57 and C(4), C(9), and C(15), respectively. The configuration of the 2-OH group was determined as α, based on the ROESY spectrum, which showed an interaction between Me(15) and H_β–C(2). Therefore, the structure of **3** was determined as (2*R*)-2-hydroxymajucin.

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh) and silica gel *H* (60 μm) were obtained from *Qingdao Haiyang Chemical Co.*, China. TLC: Precoated silica-gel GF_{254} plates (*Qingdao Haiyang Chemical Co*); detection at 254 nm, and by spraying with 5% H_2SO_4 in EtOH soln., followed by heating. Optical rotations: *Horiba SEAP-300* spectropolarimeter. IR Spectra: *Bio-Rad-FtS-135* spectrometer; KBr pellets; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker AM-400* and *DXR-500 s*pectrometers; δ in ppm, *J* in Hz. MS: *VG Autospec-3000* mass spectrometer (70 eV for EI); in *m*/*z* (rel. %).

Plant Material. The twigs and leaves of *Illicium micranthum* were collected from Wenshan county, Yunnan province, P. R. China, in September 2003. The plant material was authenticated by Prof. *Xi-Wen Li*, and a voucher specimen (No. KIB D03-10-01) was deposited at the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. Dried, powdered *I. micranthum* (3.5 kg) was extracted with EtOH/H_2O 7:3 at r.t. The resulting extract (644 g) was suspended in H_2O, and extracted first with petroleum ether and then AcOEt. The AcOEt extract (98 g) was subjected to CC (SiO_2; AcOEt/Me_2CO 1:0 → 0:1) to afford fractions *Fr. 1–4. Fr. 1* (30.1 g) was re-subjected to repeated CC (SiO_2; $CHCl_3$/Me_2CO 20:1 and 10:1,

then $CHCl_3$/MeOH 50 : 1 and 20 : 1) to afford **4** (610 mg), **5** (2.7 g), **6** (7.83 g), **8** (90 mg), and **9** (6 mg). *Fr. 2* (20.6 g) was purified by CC (SiO_2; $CHCl_3$/MeOH 15 : 1 → 9 : 1) to yield **6** (5.2 g), **7** (790 mg), **3** (18 mg), **2** (35 mg), and **1** (29 mg).

*(1*R*,2*S*)-1,2-Epoxyneomajucin* (= *(1*S*,2*R*,4*S*,6*S*,7*S*,11*R*,12*R*,15*R*)-6,11,15-Trihydroxy-2,7-dimethyl-3,9,13-trioxapentacyclo[10.3.1.0^{1,6}.0^{2,4}.0^{7,11}]hexadecane-10,14-dione*; **1**). Colorless, amorphous solid. $[\alpha]_D^{24.3} = -123.6$ ($c = 0.557$, C_5H_5N). IR (KBr): 3514, 3445, 3279, 1793, 1737. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 326 (M^+), 308 (10), 251 (26), 175 (28), 161 (46), 115 (82), 95 (47), 69 (100). HR-ESI-MS: 349.0902 ($[M+Na]^+$, $C_{15}H_{18}NaO_8^+$; calc.349.0899).

*(2*R*)-2-Hydroxyneomajucin* (= *(1*S*,2*S*,3*R*,5*R*,6*S*,10*R*,11*R*,14*R*)-3,5,10,14-Tetrahydroxy-2,6-dimethyl-8,12-dioxatetracyclo[9.3.1.0^{1,5}.0^{6,10}]pentadecane-9,13-dione*; **2**). Colorless, amorphous solid. $[\alpha]_D^{24.1} = -68.6$ ($c = 1.102$, C_5H_5N). IR (KBr): 3581, 3537, 3477, 3320, 1779, 1733. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 328 (M^+), 310 (10), 292 (17), 235 (75), 191 (37), 161 (50), 125 (74), 115 (62), 69 (100), 55 (66). HR-ESI-MS: 351.1050 ($[M+Na]^+$, $C_{15}H_{20}NaO_8^+$; calc. 351.1055).

*(2*R*)-2-Hydroxymajucin* (= *(1*S*,2*S*,3*R*,4*R*,5*R*,6*S*,10*R*,11*R*,14*R*)-3,4,5,10,14-Pentahydroxy-2,6-dimethyl-8,12-dioxatetracyclo[9.3.1.0^{1,5}.0^{6,10}]pentadecane-9,13-dione*; **3**). Colorless, amorphous solid. $[\alpha]_D^{25} = -123.4$ ($c = 1.26$, C_5H_5N). IR (KBr): 3552, 3509, 3451, 3209, 1783, 1731. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 344 (M^+), 326 (3), 308 (72), 69 (24), 251 (100), 71 (70), 69 (84), 55 (43). HR-ESI-MS: 367.1013 ($[M+Na]^+$, $C_{15}H_{20}NaO_9^+$; calc. 367.1005).

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