Rearranged Calamenene and Eudesmane Sesquiterpenoids from two Chinese Liverworts

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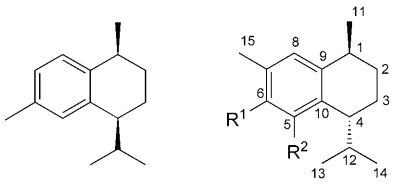
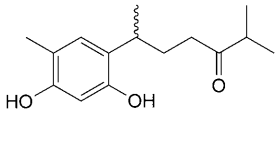
Four novel rearranged calamenene sesquiterpenoids, **1**–**4**, and two eudesmane sesquiterpenoids, **5** and **6**, were isolated from the Chinese liverworts *Chiloscyphus polyanthus* (L.) and *Bazzania japonica* S. (LAC.) LINDB. Their structures and relative configurations were determined by chemical derivatization and in-depth spectroscopic methods, especially 1D- and 2D-NMR as well as HR-MS analyses.

Introduction. – Liverworts are rich sources of terpenoids often containing pharmacologically active compounds [1][2]. Thus, many novel sesquiterpenoids have been reported from, *e.g.*, *C. polyanthus* (L.) [3][4] and *B. japonica* S. (LAC.) LINDB. [5][6], two widespread liverworts in China.

During our studies on antifungal components from Chinese liverworts [3][7][8], we isolated six new natural products. These include four sesquiterpenoids with an unprecedented rearranged calamenene skeleton, chiloscyphenol A (**1**) and B (**2**), and chiloscyphone A (**3**) from *C. polyanthus*, and bazzaniol A (**4**) from *B. japonica*, as well as two novel eudesmane sesquiterpenes, compounds **5** and **6**, from *C. polyanthus*.



	R^1	R^2
1	OH	H
1a	OAc	H
2	OH	OH
4	OMe	H



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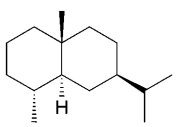
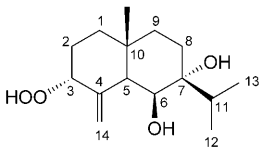


Eudesmane



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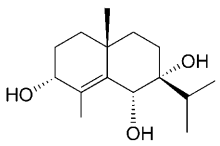

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Results and Discussion. – The molecular formula of the optically active compound **1**, $[\alpha]_D^{22} = +38.2$ ($c = 2.64$, MeOH), was found to be $C_{15}H_{22}O$, as determined by HR-EI-MS (*m*/*z* 218.1659). Four degrees of unsaturation, three Me groups at tertiary C-atoms

and one at an aromatic ring, two CH_2 and three CH groups, as well as two aromatic H-atoms suggested that **1** was a calamenene-type sesquiterpenoid. However, its ^{13}C-NMR data (*Table 1*) were somewhat different from those of *trans*- or *cis*-7-hydroxycalamenene [9], which suggested an altered substitution pattern on the aromatic ring.

Table 1. *^{1}H- and ^{13}C-NMR Data for* **1**–**4**. At 600 (^{1}H) and 150 MHz (^{13}C), resp., in $CDCl_3$; δ in ppm, J in Hz. Arbitrary atom numbering.

Position	**1**		**1a**		**2**		**3**		**4**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	2.73 (*dd*, *J*=6.6, 14.3)	32.2	2.76–2.80 (*m*)	32.3	2.72 (*dd*, *J*=6.7, 13.3)	31.5	2.84 (*d*, *J*=6.0)	30.2	2.73 (*dd*, *J*=7.0, 12.8)	32.3
2	1.32–1.39 (*m*) 1.94–1.99 (*m*)	31.1	1.37–1.42 (*m*) 1.97–2.02 (*m*)	30.6	1.17–1.25 (*m*) 1.94–1.98 (*m*)	30.0	1.51–1.55 (*m*) 1.85–1.87 (*m*)	32.1	1.31–1.37 (*m*) 1.92–1.99 (*m*)	31.1
3	1.55–1.62 (*m*) 1.83–1.88 (*m*)	21.5	1.57–1.62 (*m*) 1.86–1.90 (*m*)	20.9	1.60–1.67 (*m*) 1.68–1.73 (*m*)	21.2	2.43–2.49 (*m*) 2.58–2.61 (*m*)	37.5	1.58–1.65 (*m*) 1.83–1.90 (*m*)	21.7
4	2.68 (*dd*, *J*=6.1, 13.5)	43.6	2.71–2.73 (*m*)	43.4	2.43 (*dd*, *J*=6.0, 10.6)	41.3		217.7	2.21 (*dd*, *J*=6.6, 12.6)	44.2
5	6.68 (*s*)	114.0	6.89 (*s*)	120.6		118.9	6.40 (*s*)	103.4	6.68 (*s*)	109.8
6		151.3		146.8		149.6		152.5		155.6
7		120.7		124.5		121.6		114.9		123.8
8	7.02 (*s*)	129.3	7.12 (*s*)	129.3		129.0	6.83 (*s*)	127.9	7.00 (*s*)	129.4
9		135.4		140.4		136.6		124.0		135.0
10		139.0		138.7		138.7		153.2		138.5
11	1.29 (*d*, *J*=7.2)	22.4	1.31 (*d*, *J*=6.6)	21.2	1.27 (*d*, *J*=6.6)	21.9	1.24 (*d*, *J*=7.2)	19.1	1.27 (*d*, *J*=7.2)	22.6
12	2.20–2.25 (*m*)	31.7	2.22–2.25 (*m*)	31.4	1.46–1.49 (*m*)	31.4	2.62–2.65 (*m*)	41.0	2.22–2.25 (*m*)	32.2
13	0.75 (*d*, *J*=6.6)	17.2	0.77 (*d*, *J*=6.6)	17.0	0.48 (*d*, *J*=7.2)	18.8	1.12 (*t*, *J*=6.6)	18.3	0.75 (*d*, *J*=6.6)	17.6
14	1.04 (*d*, *J*=6.6)	21.2	1.04 (*d*, *J*=6.6)	20.6	0.59 (*d*, *J*=7.2)	21.2	1.12 (*t*, *J*=6.6)	18.3	1.04 (*d*, *J*=6.6)	21.5
15	2.26 (*s*)	15.4	2.33 (*s*)	15.6	2.28 (*s*)	16.2	2.18 (*s*)	15.1	2.21 (*s*)	16.1
MeO									3.82 (*s*)	55.2
AcO			2.18 (*s*)	19.5, 169.2						

In the HMBC spectrum of **1**, the signals at δ(H) 7.02 (*s*, H–C(8)) showed correlations with δ(C) 32.2 (C(1)) and 15.4 (C(15)), and the signal at δ(H) 6.68 (*s*, H–C(5)) correlated with δ(C) 43.6 (C(4)) (*Fig.*). In the NOESY spectrum, correlations were observed between H–C(8) and both δ(H) 2.26 (*s*, Me(15)) and 2.73 (*m*, H–C(1)), as well as between H–C(5) and both δ(H) 2.68 (*m*, H–C(4)) and 2.22 (*m*, H–C(12)). These observations suggested that compound **1** was a calamenene-type sesquiterpenoid with a rearranged Me group at position 7.

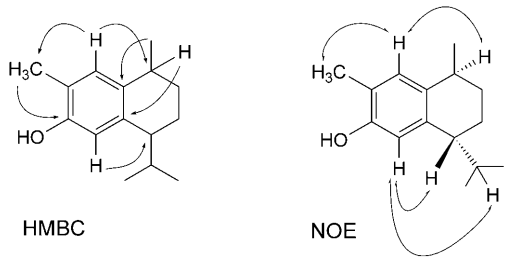

Figure. *Key HMBC and NOESY correlations for* **1**

The fact that no NOE was observed between H–C(1) and H–C(4), and analysis of the coupling constants for H–C(1) ($J(1,2\alpha)=4.8$, $J(1,2\beta)=8.7$, $J(1,11)=7.2$ Hz) and for H–C(4) ($J(4,3\alpha)=7.2$, $J(4,3\beta)=6.0$, $J(4,12)=7.2$ Hz), as determined by homodecoupled ^{1}H-NMR experiments, indicated β-orientation of the Me(11) group and α-orientation of the i-Pr group [9]. We tried to obtain suitable crystals for X-ray crystallography by transforming **1** into its acetate **1a** (M^+ at m/z 260.1742) or the corresponding methyl ether **4** (M^+ at 232.1870). However, all crystallization attempts were unsuccessful. In the NOESY spectra of **1a** and **4**, the Ac and MeO H-atoms exhibited NOEs with H–C(5) and Me(15), which further confirmed the above conclusions. From the above results, the structure of **1** was, thus, established as (5*S**,8*R**)-5,6,7,8-tetrahydro-3,5-dimethyl-8-(1-methylethyl)naphthalen-2-ol, and the compound was named *chiloscyphenol A*.

Compound **2**, a yellowish, optically active oil ($[\alpha]_D^{23}=+98.5$ ($c=0.50$, MeOH)), was determined to have the molecular formula $C_{15}H_{22}O_2$ (m/z 234.1605). The ^{1}H- and ^{13}C-NMR data of **2** (*Table 1*) were similar to those of **1**, except for one more OH substituent at the benzene ring, as confirmed by only one aromatic ^{1}H-NMR resonance at δ(H) 7.06 (*s*). HMBC and NOESY experiments showed that compound **2** was also a rearranged calamenene-type sesquiterpenoid. Its structure was determined as (5*S**,8*R**)-5,6,7,8-tetrahydro-3,5-dimethyl-8-(1-methylethyl)naphthalen-1,2-diol, and it was named *chiloscyphenol B*.

Compound **3** had the molecular formula $C_{15}H_{21}O_3$ (m/z 249.1480). The ^{1}H-NMR spectrum (*Table 1*) displayed the signals of two aromatic H-atoms, three Me groups at tertiary C-atoms, one Me group on an aromatic ring, and two CH_2 moieties. From the DEPT spectrum of **3**, the 15 carbon signals could be assigned to four Me, two CH_2, two CH, and seven quaternary C-atoms, including one C=O signal at δ(C) 217.7 (C(4)). The Me group on the aromatic ring was at C(7) according to a NOESY experiment. Thus, the structure of **3** was determined as 6-(2,4-dihydroxy-5-methylphenyl)-2-methylheptan-3-one, and the compound was named *chiloscyphone A*.

Compound **4**, a yellowish, optically active oil ($[\alpha]_D^{22}=+98.5$ ($c=0.50$, MeOH)), was found to have the molecular formula $C_{16}H_{24}O$ (m/z 232.1870). The ^{1}H- and ^{13}C-NMR spectroscopic data were identical to those of the synthesized methyl ether derivative obtained from **1**. Thus, the structure of **4** was identified as (1*S**,4*R**)-1,2,3,4-tetrahydro-1,7-dimethyl-4-(1-methylethyl)-6-methoxynaphthalene.

Note that compounds **1**–**4** are the first examples of sesquiterpenoids with a *rearranged* calamenene skeleton isolated from liverworts.

The ESI mass spectrum of compound **5** exhibited the molecular-ion peak at *m*/*z* 270.4. In the HR-EI mass spectrum, the $[M-H_2O_2]^+$ peak appeared at *m*/*z* 236.1801 ($C_{15}H_{24}O_2^+$; calc. 236.1776). So, the elemental composition of **5** was determined as $C_{15}H_{26}O_4$. An eudesmane skeleton was derived from MS, and 1D- and 2D-NMR data (*Table 2*). The olefinic signals at δ(C) 145.26 (C(4)) and 114.86 (C(14)) suggested $\Delta^{4(14)}$ unsaturation, as confirmed by two vinylic signals in the ^{1}H-NMR spectrum at δ(H) 5.28 (s) and 5.35 (*s*). A DEPT experiment showed two CH signals at δ(C) 86.66 and 71.50 as well as one quaternary carbon at δ(C) 74.94, indicating the presence of three oxygenated C-atoms. A 7-OH group was inferred from HMBC correlations between a pair of Me *doublet*s at δ(H) 0.94 and 0.97 (Me(12) and Me(13)) with δ(C) 74.94 (C(7)). The signal at δ(H) 3.99, corresponding to δ(C) 71.50 according to the HMQC spectrum, showed HMBC correlations to both δ(C) 74.94 and 145.26 (C(4)). The signal at δ(H) 71.50, bearing another OH group, was assigned to C(6). So, the other two O-atoms required a hydroperoxide (OOH) function, which was placed at C(3), its α-orientation being derived from the small coupling constant for H–C(3) at δ(H) 4.43 (*t*, $J=3.0$ Hz). Furthermore, the β-configuration of the 6-OH group was deduced from a NOESY cross-peak between δ(H) 2.74 (*d*, $J=1.8$ Hz, H–C(5)) and δ(H) 3.99 (*s*, H–C(6)). Finally, the 7-OH group at C(7) was assumed to be α-oriented by analogy to known eudesmanes [10].

From the above data, compound **5** was identified as 3α-hydroperoxyeudesm-4(14)-ene-6β,7α-diol (=(1*S**,2*R**,4a*R**,7*R**)-decahydro-7-hydroperoxy-4a-methyl-2-(1-methylethyl)-8-methylidenenaphthalene-1,2-diol).

Table 2. *^{1}H- and ^{13}C-NMR Data of* **5** *and* **6**. At 600 (^{1}H) and 150 MHz (^{13}C), resp.; δ in ppm, *J* in Hz. Arbitrary atom numbering.

Position	**5**[a])		**6**[b])	
	^{1}H	^{13}C	^{1}H	^{13}C
1	1.16–1.19 (*m*)	38.00	1.48–1.51 (*m*)	35.23
	1.58–1.60 (*m*)		1.04–1.06 (*m*)	
2	1.89–1.92 (*m*)	25.91	1.71–1.74 (*m*)	28.00
			1.57–1.61 (*m*)	
3	4.43 (*t*, $J=3.0$)	86.67	3.64 (br. *s*)	68.39
4		145.26		131.49
5	2.74 (*d*, $J=1.8$)	42.76		138.25
6	3.99 (*s*)	71.50	4.32 (*d*, $J=3.0$)	68.44
7		74.94		73.96
8	1.60–1.63 (*m*)	26.94	1.60–1.65 (*m*)	26.17
	1.75–1.77 (*m*)		1.38–1.42 (*m*)	
9	1.35–1.39 (*m*)	35.61	1.52–1.57 (*m*)	36.33
	1.58–1.60 (*m*)		1.18 (*d*, $J=6.6$)	
10		34.70		33.21
11	2.00–2.02 (*m*)	32.52	1.82–1.89 (*m*)	32.70
12/13	0.94/0.97 (2*d*, $J=6.6$)	15.77/15.84	0.85/0.87 (2*d*, $J=6.6$)	16.44/16.28
14	5.28, 5.35 (2 br. *s*)	114.86	1.70 (*s*)	17.03
15	1.70 (*s*)	19.26	1.07 (*s*)	25.71

[a]) In $CDCl_3$. [b]) In (D_6)DMSO.

Compound **6** was obtained as a colorless oil with the molecular formula $C_{15}H_{26}O_3$, as deduced by ESI-MS (M^+ at m/z 254.7) and HR-EI-MS ($[M-H_2O]^+$ at m/z 236.1833). Four Me groups and a tetrasubstituted C=C bond were evident in the NMR spectra (*Table 2*), which also suggested an eudesmane skeleton. Furthermore, the carbon signals at δ(C) 68.39, 68.44, and 73.96 indicated two secondary and one tertiary O-bearing C-atoms. This was in good agreement with the ^{1}H-NMR data of **6**, which exhibited two carbinol H-atoms at δ(H) 3.64 (br. *s*, H–C(3)) and 4.32 (*d*, $J=3.0$, H–C(6)). These data clearly pointed to an eudesmane skeleton with $\Delta^{4,5}$ unsaturation and OH groups in positions 3, 6, and 7. The relative α-configuration of the 3-OH group was deduced by a NOESY correlation between δ(H) 3.64 and 1.49 (H_β–C(1)). The 6-OH and 7-OH functions were both assumed to be α-configured due to NOEs between Me(15) (δ(H) 1.07 (*s*)) and both H–C(11) (δ(H) 1.86 (*m*)) and H–C(6).

From the above data, the structure of compound **6** was identified as eudesm-4-ene-3α,6α,7α-triol (=(2*R**,4a*R**)-1,2,3,4,4a,5,6,7-octahydro-4a,8-dimethyl-2-(1-methylethyl)naphthalene-1,2,7-triol).

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Experimental Part

TLC was carried out on silica-gel-precoated glass plates (*Kieselgel 60 F_{254}*; *Merck*) eluting with petroleum ether (PE)/Me_2CO 1:1, 2:1, and 3:1, or with $CHCl_3$/MeOH 10:1. Silica gel *60* (70–230 μm; *Merck*) was employed for normal-phase column chromatography (CC). Reverse-phase CC was performed on *Sephadex LH-20* with $CHCl_3$/MeOH 1:1. Optical rotations were measured on a *Perkin-Elmer 341* polarimeter at 589 nm and 20°. ^{1}H- and ^{13}C-NMR Spectra were recorded at 600 and 150 MHz, resp., on a *Bruker AVANCE-600* spectrometer in $CDCl_3$ and (D_6)DMSO; chemical shifts δ in ppm rel. to residual solvent peaks (δ(H) 7.24, δ(C) 77.0 for $CDCl_3$; δ(H) 2.50, δ(C) 39.8 for (D_6)DMSO). HR EI-MS was performed on a *Waters GCT* system using He (60 kPa, 1 ml/min) as carrier gas. Samples were analyzed on a *HP-5* column (15 m × 0.25 mm; 0.25 μm film). ESI-MS Data were recorded on an *API-4000* mass spectrometer; in m/z.

Plant Material. Chiloscyphus polyanthus was gathered at Mount Tai, Shandong, China, in August 2003. *Bazzania japonica* was collected at Shiwan Mountains, Guangxi, China, in May 2001. The plants were identified by *Rui-Liang Zhu*, and voucher specimens were deposited at the School of Pharmaceutical Sciences, Shandong University, China.

Extraction and Isolation. The EtOH extract of *C. polyanthus* (78 g) was separated into 18 fractions (Fr.) by CC (SiO_2; $CHCl_3$/MeOH). *Fr. 5*, eluted with $CHCl_3$/MeOH 85:1, was subjected to RP-CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1) to afford **1** (35.0 mg), **3** (15.2 mg), and **5** (7.5 mg). *Fr. 11* and *Fr. 16*, eluted with $CHCl_3$/MeOH 60:1, were rechromatographed (1. SiO_2, $CHCl_3$/MeOH; 2. *Sephadex LH-20*) to provided **2** (17.6 mg) and **6** (10.5 mg).

The Et_2O extract (12 g) of *B. japonica* was subjected to CC (SiO_2; PE/Me_2CO gradient) to yield eleven fractions. *Fr. 8*, eluted with PE/Me_2CO 30:1, was purified by RP-CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1), which yielded nine subfractions (*Fr. 8.1–8.9*). *Fr. 8.4* (320 mg) was further separated by CC (SiO_2; PE/Me_2CO gradient) to afford **4** (15 mg).

Derivatization of **1**. a) *Methylation*. To a soln. of **1** (8 mg) in $CDCl_3$ (2 ml), diazomethane (2 ml) was added, and the mixture was stirred at 20° for 5 h. The resulting soln. was concentrated *in vacuo* to afford **4** (4.2 mg). The anal. data of semi-synthetic **4** were identical with those of the purified, natural sample.

b) *Esterification*. To a soln. of **1** (10 mg) in $CDCl_3$ (3 ml) was added Ac_2O (1 ml), and the mixture was stirred at 20° for 4 h. The org. phase was washed with H_2O (3×) and then evaporated in *vacuo* to afford the acetate **1a** (5.6 mg) as a colorless oil. HR-EI-MS: 260.1742 (M^+, $C_{17}H_{24}O_2^+$; calc. 260.1776).

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