Terpenoids from the Chinese Liverwort *Chiloscyphus polyanthus*

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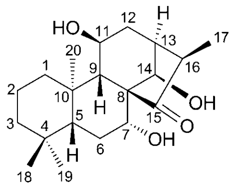
A new diterpene, (7α,11β,14β,16*R*)-7,11,14-trihydroxy-*ent*-kaur-15-one (**1**), and a new sesquiterpene, polyanthuslide (**2**), were isolated from the Chinese liverwort *Chiloscyphus polyanthus*. Their structures were determined on the basis of extensive spectroscopic analyses, and the configuration of **2** was established by X-ray crystallographic analysis.

Introduction. – Bryophytes have been preferential natural sources for the search of alternative pharmacological and agricultural medicines, particularly antifungal agents [1][2]. In the past few decades, a number of lipophilic terpenoids have been isolated from the bryophyte gametophytes which constitute a rich source of bioactive molecules [3–5]. The liverwort *Chiloscyphus polyanthus* has been investigated phytochemically, and several eudesmane sesquiterpenes have been obtained from this species [6][7]. As a result of our ongoing research on antifungal agents from bryophytes, we isolated a new diterpene, (7α,11β,14β,16*R*)-7,11,14-trihydroxy-*ent*-kaur-15-one (**1**), and a new sesquiterpene, polyanthuslide (**2**), from this plant.



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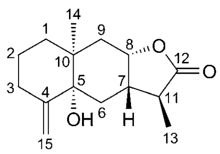

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Result and Discussion. – The EtOH extract of the liverwort gametophyte of *C. polyanthus* was fractionated and purified by repeated column chromatography (silica-gel and *Sephadex LH-20*) to afford two new terpenes **1** and **2**.

(7α,11β,14β,16*R*)-7,11,14-Trihydroxy-*ent*-kaur-15-one (**1**), obtained as a white, amorphous powder, possesses the molecular formula $C_{20}H_{32}O_4$ according to the HR-ESI-MS (*m*/*z* 359.2206 ([M+Na]$^+$; calc. 359.2198)), indicating five degrees of unsaturation. The IR spectrum exhibited a strong CO absorption (1723 cm^{-1}) and absorption of OH groups (3317 cm^{-1}). The ^{1}H-NMR (*Table 1*) spectrum of **1** showed signals for three tertiary Me groups (δ(H) 0.86 (*s*), 0.93 (*s*), and 1.02 (*s*)), one secondary Me group (δ(H) 1.31, *d*, J = 6.6), and three O-bearing CH groups (δ(H) 3.91 (*d*, J = 6.0), 4.28 (*dd*, J = 12.0, 3.6), and 4.93 (br. *s*)).

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1**. At 600 and 150 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H_α–C(1)	1.79–1.83 (*m*)	39.3	C(10)		38.5
H_β–C(1)	0.89–0.92 (*m*)		H–C(11)	3.91 (*d*, J = 6.0)	64.3
H_α–C(2)	1.61–1.66 (*m*)	18.4	H_α–C(12)	1.92–1.95 (*m*)	33.3
H_β–C(2)	1.46–1.51 (*m*)		H_β–C(12)	2.06 (br. *d*, J = 15.6)	
H_α–C(3)	1.42–1.47 (*m*)	41.5	H–C(13)	2.43–2.48 (*m*)	42.0
H_β–C(3)	1.14–1.21 (*m*)		H–C(14)	4.93 (br. *s*)	75.1
C(4)		33.6	C(15)		220.6
H–C(5)	0.99 (*dd*, J = 12.0, 1.8)	53.7	H–C(16)	2.98–3.03 (*m*)	44.6
H_α–C(6)	1.70 (*q*, J = 12.0)	28.0	Me(17)	1.31 (*d*, J = 6.6)	10.5
H_β–C(6)	1.95–1.99 (*m*)		Me(18)	0.93 (*s*)	33.4
H–C(7)	4.28 (*dd*, J = 12.0, 3.6)	75.8	Me(19)	0.86 (*s*)	21.7
C(8)		59.4	Me(20)	1.02 (*s*)	18.1
H–C(9)	1.18 (br. *s*)	65.5			

The ^{13}C-NMR spectrum (*Table 1*) exhibited 20 C-atom signals, which were assigned, by chemical-shift values and HMQC spectrum, to four Me, five CH_2, and seven CH groups (three O-bearing), and four quaternary C-atoms (one ketone C-atom). Further 2D-NMR studies including ^{1}H,^{1}H-COSY (*Fig. 1*), HMQC, and HMBC (*Fig. 1*) experiments provided the gross structure of **1** as a kaurane-type diterpenoid or its enantiomer [8] with a ketone and three OH groups. The HMBC of Me(17) to C(15) (δ(C) 220.6) indicated the ketone group C(15)=O. The HMBC of H–C(14) (δ(H) 4.93 (br. *s*)) to C(15) and C(16) suggested that a OH group is present at C(14). The ^{1}H,^{1}H-COSY $H_{\alpha,\beta}$–C(6)/H–C(7) (δ(H) 4.28 (*dd*, J = 12.0, 3.6)), H–C(9)/H–C(11) (δ(H) 3.91 (*d*, J = 6.0)), and $H_{\alpha,\beta}$–C(12)/H–C(11) indicated that the remaining two OH groups are at C(7) and C(11), respectively.

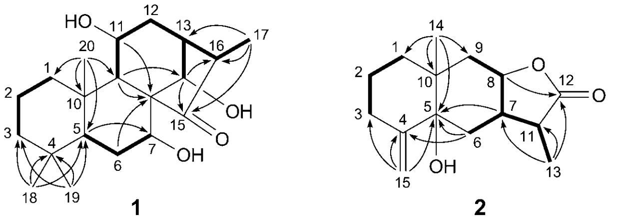
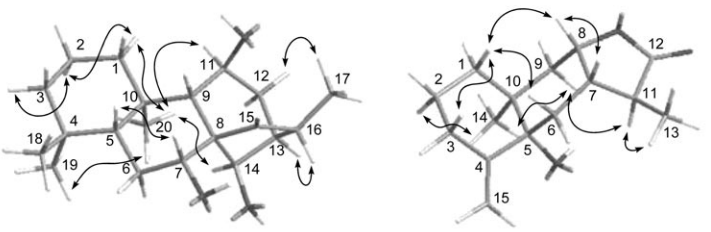


Fig. 1. *Key ^{1}H,^{1}H-COSY* (—) *and HMBC* (H → C) *correlations of* **1** *and* **2**

The relative configuration of **1** was established through its NOESY spectrum. The NOE correlations (*Fig. 2*) H–C(7)/H–C(5), H–C(11)/Me(20), H–C(14)/Me(20), and H–C(16)/H–C(13) indicated the OH group at C(7) is α-oriented, and the OH groups at C(11) and C(14), and the Me group at C(16) are β-oriented, which was supported by the chemical shifts and splitting patterns of corresponding H-atoms [8][9].

The absolute configuration of **1** was determined by the CD spectrum that displayed first a negative ($\Delta\varepsilon_{308}$ – 0.33) and then a positive ($\Delta\varepsilon_{276}$ + 0.23) *Cotton* effect. The negative *Cotton* effect of **1** with the saturated ketone system was analogous to that of (16*R*)-*ent*-kaur-15-one (λ_{max} 314 nm) and (6α,11β,16*R*)-6,11-dihydroxy-*ent*-kaur-15-



1 **2**

Fig. 2. *Key NOESY* (H ↔ H) *correlations of* **1** *and* **2**

one (λ_{max} 307 nm) [10]. Accordingly, **1** was determined as (7α,11β,14β,$16R$)-7,11,14-trihydroxy-*ent*-kaur-15-one.

Polyanthuslide (**2**) was obtained as colorless crystals from an aqueous MeOH solution. The molecular formula was determined as $C_{15}H_{22}O_3$ on the basis of HR-ESI-MS (*m*/*z* 273.1474 ($[M+Na]^+$; calc. 273.1466)), indicating five degrees of unsaturation. The ^{1}H-NMR (*Table 2*) spectrum of **2** indicated the presence of one tertiary Me group (δ(H) 0.90), one secondary Me group (δ(H) 1.21, *d*, $J = 7.0$), one O-bearing CH group (δ(H) 4.73 (*dt*, $J = 11.5$, 6.6)), and two olefinic H-atoms (δ(H) 4.91 and 4.99, each br. *s*). According to chemical-shift values and HMQC spectrum, 15 signals in the ^{13}C-NMR spectrum (*Table 2*) of **2** were ascribed to two Me, six CH_2 (one olefinic), three CH groups (one O-bearing), and four quaternary C-atoms (one olefinic, one O-bearing, and one ester or lactone C-atom). These data suggested that **2** possesses the features of a bicyclic sesquiterpenoid of eremophilane-, lemnalane-, eudesmane-, carotane-, or mexicanane-type.

Table 2. *^{1}H- and ^{13}C-NMR Data of* **2**. At 600 and 150 MHz, resp., in $CDCl_3$; δ in ppm, *J* in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H_α–C(1)	1.37–1.42 (*m*)	34.5	H–C(8)	4.73 (*dt*, $J = 11.5$, 6.6)	75.2
H_β–C(1)	1.61–1.63 (*m*)		H_α–C(9)	1.84 (*dd*, $J = 13.5$, 11.5)	37.6
H_α–C(2)	1.59–1.63 (*m*)	21.8	H_β–C(9)	1.73 (*dd*, $J = 13.5$, 6.3)	
H_β–C(2)	1.76–1.81 (*m*)		C(10)		40.2
H_α–C(3)	2.37–2.41 (*m*)	33.0	H–C(11)	3.24–3.29 (*m*)	37.6
H_β–C(3)	2.28–2.30 (*m*)		C(12)		180.2
C(4)		152.3	Me(13)	1.21 (*d*, $J = 7.0$)	13.3
C(5)		76.8	Me(14)	0.90 (*s*)	21.7
H_α–C(6)	1.68 (*d*, $J = 15.4$)	31.4	H_a–C(15)	4.99 (br. *s*)	108.4
H_β–C(6)	2.34 (*dd*, $J = 15.4$, 6.8)		H_b–C(15)	4.91 (br. *s*)	
H–C(7)	2.25–2.28 (*m*)	41.3			

On the basis of the 2D-NMR data, including ^{1}H,^{1}H-COSY (*Fig. 1*), HMQC, and HMBC (*Fig. 1*), compound **2** was identified as an eudesmane-type sesquiterpenoid with the same constitutional formula as 11α,13-dihydrotelekin [11]. However, their ^{1}H- and ^{13}C-NMR data were quite different as a result of the different relative configurations.

The relative configuration of **2** was determined by its NOESY spectrum. The NOE correlations (*Fig. 2*) H_β–C(3)/H_β–C(1), H_β–C(1)/H_β–C(6), H_β–C(1)/H–C(8), and H–C(8)/H–C(7) indicated that they were on the same side of the molecule, and they were arbitrarily assigned as β-oriented. The correlations H_α–C(2)/Me(14), Me(14)/H_α–C(9), and H_α–C(9)/H–C(11) indicated α-orientation. Thus, **2** is a rare natural *cis*-eudesmane derivative [12]. The absolute configuration of **2** was determined as depicted by a single-crystal X-ray diffraction analysis with CuK_α radiation (*Fig. 3*).

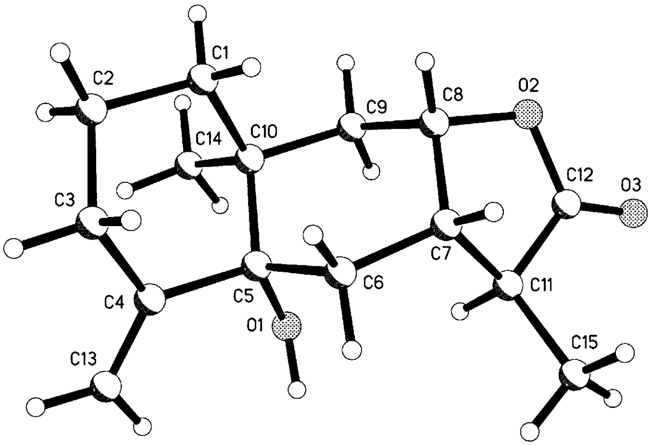


Fig. 3. *Single-crystal X-ray structure of* **2**

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Experiment Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, 10–40 mm; *Qingdao Haiyang Chemical Co. Ltd.*, P. R. China) and *Sephadex LH-20* (*Amersham Biosciences*). TLC: silica gel *GF254* plates; visualization by heating the plates sprayed with 10% H_2SO_4/EtOH. M.p.: *X-6* melting point apparatus (*Beijing TECH Instrument Co. Ltd.*, P. R. China). Optical rotations: *Gyromat-Hp* digital automatic polarimeter. UV: *Agilent 8453E* UV-Visible spectroscopy system. IR: *Thermo Nicolet NEXUS 470* FT-IR spectrometer. 1D- and 2D-NMR spectra: *Bruker Avance DRX-600* spectrometer at 600 (^{1}H) and 150 (^{13}C) MHz, with TMS as internal standard. ESI-MS: *API 4000* triple-stage quadrupole mass spectrometer; HR-ESI-MS: *LTQ-Orbitrap XL* instrument.

Plant Material. The liverwort *C. polyanthus* (L.) Corda was collected in June 2003 in Mount Yue-lu, Hunan Province, P. R. China, and identified by Prof. *C. Gao*, Shenyang Institute of Applied Ecology, Chinese Academy of Sciences. A voucher specimen (No. 20030626CP) has been deposited with the Laboratory of Natural Products Chemistry, School of Pharmaceutical Sciences, Shandong University, P. R. China.

Extraction and Isolation. The air-dried, powdered liverwort material (1.2 kg) was extracted four times with 95% EtOH under reflux. The combined, evaporated EtOH extracts (75 g) were subjected to CC (SiO_2; petroleum ether (PE)/acetone gradient) to furnish eleven fractions. *Fr. 2* eluted with PE/acetone 93 : 7 was further purified by CC (SiO_2; PE/AcOEt 8 : 2) to afford **2** (40 mg). *Fr. 3* eluted with PE/acetone 10 : 90 was further purified by repeated CC (SiO_2; PE/AcOEt) to yield **1** (7 mg).

*(7α,11β,14β,16*R*)-7,11,14-Trihydroxy-*ent*-kaur-15-one* (=*(5β,7α,8α,9β,10α,11β,13α,14*R*,16β)-7,11,14-Trihydroxykauran-15-one*; **1**). White, amorphous powder. $[\alpha]_D^{20} = -98.3$ ($c = 0.21$, MeOH). UV (MeOH): 202 (3.02). CD (MeOH): $\Delta\varepsilon_{308} - 0.33$, $\Delta\varepsilon_{276} + 0.23$. IR (KBr): 3317, 2929, 1723, 1466, 1387, 1370, 1094, 1069, 1024. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 711 (4, $[2M+K]^+$), 695 (15, $[2M+Na]^+$), 673 (56, $[2M+1]^+$), 375 (6, $[M+K]^+$), 359 (9, $[M+Na]^+$), 354 (100, $[M+NH_4]^+$), 337 (5, $[M+1]^+$). HR-ESI-MS: 359.2206 ($[M+Na]^+$, $C_{20}H_{32}NaO_4^+$; calc. 359.2198).

Polyanthuslide (=*(3*S*,3a*S*,4a*R*,8a*S*,9a*S*)-Decahydro-4a-hydroxy-3,8a-dimethyl-5-methylidenenaphtho[2,3-*b*]furan-2(3*H*)-one*; **2**). Colorless needles (MeOH/H_2O). M.p. 135–136°. $[\alpha]_D^{20} = -40.0$ ($c = 0.11$, MeOH). UV (MeOH): 202 (3.56). CD (MeOH): $\Delta\varepsilon_{197} + 7.89$. IR (KBr): 3459, 2931, 1762, 1160, 1042, 987, 908. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 523 (9, $[2M+Na]^+$), 501 (46, $[2M+1]^+$), 273 (17, $[M+Na]^+$), 268 (100, $[M+NH_4]^+$), 251 (8, $[M+1]^+$). HR-ESI-MS: 273.1474 ($[M+Na]^+$, $C_{15}H_{22}NaO_3^+$; calc. 273.1466).

X-Ray Crystallographic Analysis of Polyanthuslide (**2**). Single crystals suitable for X-ray analysis were obtained by recrystallization from an aq. MeOH soln. A colorless prismatic crystal with dimensions of *ca.* $0.34 \times 0.32 \times 0.22$ mm^3 was used for analysis. All measurements were conducted on a *Rigaku MM-002* diffractometer employing graphite-monochromated CuK_α radiation ($\lambda = 1.54187$ Å) at 113 K and operating in the ω-scan mode. Crystal data: $C_{15}H_{22}O_3$, M_r 250.33, monoclinic, space group $P12_11$, $a = 9.6762(19)$, $b = 11.924(2)$, $c = 12.260(3)$ Å, $V = 1348.4(5)$ Å^3, $Z = 4$, $D_{calc.} = 1.233$ Mg/m^3, $F(000) = 544$, and $\mu(\mathrm{Cu}K_\alpha) = 0.675$ mm^{-1}. The structure was solved by the direct method SHELXS-97 and refined with full-matrix least-squares calculations on F^2 using SHELXL-97 [13]. A total of 17900 reflections (4721 unique, $R_{int} = 0.0429$) were collected from the θ range of 3.78 to 72.42°. All non-H-atoms were given anisotropic thermal parameters. The H-atom positions were geometrically idealized. The final stage converged to $R_1 = 0.0343$ ($wR_2 = 0.0849$) for 4721 observed reflections (with $I > 2\sigma(I)$) and 338 variable parameters, $R_1 = 0.0358$ ($wR_2 = 0.0900$) for all unique reflections and GOF = 1.103. Crystallographic data for **2** have been deposited with the *Cambridge Crystallographic Data Centre* (deposition No. CCDC-768067). Copies of the data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/conts/retrieving.html or from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk).

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