A New Tricyclo[6.3.1.0^{2,5}]dodecane Sesquiterpene from Cultures of the Basidiomycete *Campanella junghuhnii*

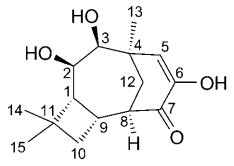
by **Rong Liu**[a]), **Zhong-Yu Zhou**[a]), **Di Xu**[a])[b]), **Fei Wang**[a]), and **Ji-Kai Liu***[a])

[a]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China (phone: +86-871-521-6327; fax: +86-871-515-0227; e-mail: jkliu@mail.kib.ac.cn)

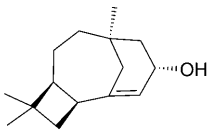
[b]) South China Agricultural University, Guangzhou 510642, P. R. China

A new sesquiterpene with a tricyclo[6.3.1.0^{2,5}]dodecane skeleton, 2,3,6-trihydroxycaryol-5-en-7-one (**1**), was isolated from the culture of the basidiomycete *Campanella junghuhnii*. The structure of **1** was elucidated on the basis of extensive spectroscopic analysis including IR, UV, MS, 1D- and 2D-NMR experiments.

Introduction. – *Campanella junghuhnii*, belonging to the family Marasmiaceae, is a small, thin, white mushroom, usually growing on bamboo stems [1]. So far, secondary metabolites produced by fungi of the genus *Campanella* have not been reported. As one part of our research for naturally occurring bioactive metabolites from higher fungi in China [2–6], we have carried out a chemical investigation on the cultures of *C. junghuhnii* which led to the isolation of a new sesquiterpene (**1**). Comparison of the NMR data of **1** with those of the cytotoxic sesquiterpene caryol-7-en-6-ol (**2**) [7], which was isolated from a New Zealand sponge of the genus *Eurypon*, implied that they share the same tricyclo[6.3.1.0^{2,5}]dodecane skeleton. This is the first report of the isolation of a sesquiterpene with this skeleton from a higher fungus. The structural elucidation of **1** was mainly performed with 1D- and 2D-NMR experiments.[1])



1[1])



2

Results and Discussion. – The culture of *C. junghuhnii* (20 l) was filtered, and the filtrate was extracted four times with AcOEt. The organic layer was concentrated *in vacuo* to give a crude extract (40 g), which was subjected to repeated column chromatography to afford pure **1**.

[1]) Arbitrary numbering. For the systematic name, see *Exper. Part.*

Compound **1** was obtained as a colorless oil. The HR-ESI-MS exhibited a *quasi*-molecular ion peak at m/z 289.1420 ($[M+Na]^+$; calc. 289.1415), indicating the molecular formula $C_{15}H_{22}O_4$. The IR spectrum showed absorption bands for OH (3422 cm^{-1}), C=O (1734 cm^{-1}), and C=C (1667 cm^{-1}) functional groups. Based on the UV absorption maximum at 277 nm, and the C=O signal at δ(C) 198.2 (*s*, C(7)) and C=C signals at δ(C) 123.5 (*d*, C(5)[1])) and δ(C) 146.3 (*s*, C(6)) in the ^{13}C-NMR spectrum (*Table*), it was concluded that the C=O group was present as a *α,β*-unsaturated ketone group. Broad-band decoupled ^{13}C-NMR and DEPT spectra disclosed the presence of three Me, two CH_2, and six CH groups (thereof two O−CH groups at δ(C) 70.1 (*d*, C(2)) and δ(C) 81.6 (*d*, C(3))), and four quaternary C-atoms. Comparing the ^{13}C-NMR data of **1** with those of **2**, the five degrees of unsaturation of **1** required by the molecular formula could be accommodated by the presence of an enone group and of a tricyclic skeleton.

The ^{1}H,^{1}H-COSY spectrum of **1** revealed two spin systems: the C(3)−C(2)−C(1)−C(9)−C(8)−C(12) and the C(9)−C(10) unit (see the formula for the arbitrary numbering system). C(2) and C(3) were both substituted by OH groups as deduced from the NMR signals (*Table*) at δ(H) 4.18 (*dd*, $J=11.6$, 1.2, H−C(2)), δ(C) 70.1 (*d*, C(2)), δ(H) 3.68 (*d*, $J=1.2$, H−C(3)), δ(C) 81.6 (*d*, C(3)) and the HMBC of H−C(2) with C(1), C(3), C(9), and C(11), and H−C(3) with C(1), C(2), C(4), C(5), and C(13) (*Fig. 1*). The HMBC spectrum showed also correlations from H−C(5), H−C(8), H−C(9), and H−C(12) to the ketone C=O group at δ(C) 198.2 (*s*, C(7)), which indicated that the ketone C=O group is located at C(7) and the C=C bond at C(5)/C(6). Furthermore, an oxygenated olefinic quaternary C-atom (δ(C) 146.3) assigned to C(6) was supported by the HMBCs from H−C(8) to C(6), and from H−C(12) and H−C(13) to C(5). The relative configuration of **1** was determined by a ROESY experiment. The ROESY correlations (*Fig. 2*) of H−C(3) and H−C(5) with α-Me(13), H−C(8) and Me(15) with H_α−C(10), H−C(2) with α-Me(15), H−C(1) and H−C(9) with H_β−C(12), and Me(14) with H_β−C(10) indicated that

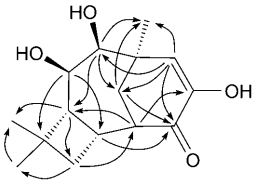


Fig. 1. *Key HMBC data of* **1**

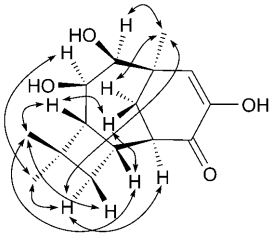


Fig. 2. *Key ROESY correlations of* **1**

H–C(1), H–C(2), H–C(3), H–C(5), H–C(8), H–C(9), Me(14), and Me(15) possessed β-, α-, α-, α-, β-, β-, and α-orientations, respectively. On the basis of the above evidence, the structure of **1** was deduced as 2,3,6-trihydroxycaryol-5-en-7-one.

Table. *NMR Spectral Data of* **1** *and* **2**. Measured in $CDCl_3$; δ in ppm, J in Hz.

	1		**2**	
	δ(H)[a])	δ(C)[b])	δ(H)[c])	δ(C)[d])
H–C(1)	2.46 (*dd*, *J* = 11.6, 11.6)	47.6 (*d*)	1.82 – 1.89 (*m*)	49.9 (*d*)
H–C(2) or CH_2(2)	4.18 (*dd*, *J* = 11.6, 1.2)	70.1 (*d*)	1.58 – 1.65 (*m*), 1.42 – 1.50 (*m*)	22.2 (*t*)
H–C(3) or CH_2(3)	3.68 (*d*, *J* = 1.2)	81.6 (*d*)	1.20 – 1.29 (*m*), 1.12 – 1.19 (*m*)	37.9 (*t*)
C(4)		37.7 (*s*)		33.5 (*s*)
H–C(5) or CH_2(5)	5.84 (*s*)	123.5 (*d*)	1.96 (*dd*, *J* = 13.0, 9.3), 0.08 – 1.03 (*m*)	47.2 (*t*)
C(6) or H–C(6)		146.3 (*s*)	4.45 – 4.51 (*m*)	67.8 (*d*)
C(7) or H–C(7)		198.2 (*s*)	5.36 – 5.43 (*m*)	125.3 (*d*)
H–C(8) or C(8)	2.59 – 2.66 (*m*)	44.1 (*d*)		140.8 (*s*)
H–C(9)	2.80 – 2.89 (*m*)	32.3 (*d*)	3.21 – 3.30 (*m*)	38.3 (*d*)
CH_2(10)	1.72 – 1.78 (*m*), 1.57 (*dd*, *J* = 12.3, 9.7)	35.5 (*t*)	1.97 (*t*, *J* = 10.7), 1.67 – 1.74 (*m*)	36.0 (*t*)
C(11)		32.4 (*s*)		34.7 (*s*)
CH_2(12)	2.14 (*d*, *J* = 5.3), 1.82 (*dd*, *J* = 6.5, 5.3)	32.8 (*t*)	2.21 – 2.28 (*m*), 1.36 (br. *d*, *J* = 12.5, 2.0)	35.2 (*t*)
Me(13)	1.22 (*s*)	28.9 (*q*)	1.05 (*s*)	28.2 (*q*)
Me(14)	1.18 (*s*)	31.2 (*q*)	0.96 (*s*)	24.2 (*q*)
Me(15)	0.93 (*s*)	25.3 (*q*)	1.22 (*s*)	30.3 (*q*)

[a]) Recorded at 400 MHz. [b]) Recorded at 125 MHz. [c]) Recorded at 300 MHz. [d]) Recorded at 75 MHz; multiplicities inferred from DEPT and HMQC experiments.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200 – 300 mesh, *Qingdao Marine Chemical Inc.*, P. R. China) and *Sephadex LH-20* (*Amersham Biosciences*, Uppsala, Sweden); TLC monitoring, visualization by heating the SiO_2 plates sprayed with 10% H_2SO_4 in EtOH. Optical rotations: *Horiba SEPA-300* digital polarimeter. UV Spectra: *Shimadzu UV-210* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Bruker Tensor-27* spectrometer; with KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers; δ in ppm, J in Hz. MS: *VG Autospec-3000* and *API QSTAR-Pulsar-1* spectrometer; in *m*/*z* (rel. int.).

Mushroom Material and Culture. The fungus *C. junghuhnii* was isolated from the tissue culture of its fruiting bodies collected at Gaoligong Mountains, Yunnan Province, P. R. China, in July 2006, and identified by Prof. *Mu Zang*, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). The voucher specimen was deposited with the Herbarium of the Kunming Institute of Botany, CAS. Culture medium: potato (peeled) 200 g, glucose 20 g, KH_2PO_4 3 g, $MgSO_4$ 1.5 g, citric acid 0.1 g, and thiamine hydrochloride 10 mg in 1 l of deionized H_2O. The pH was adjusted to 6.5 before autoclaving, and the fermentation was carried out on a shaker at 25° and 150 rpm for 20 d.

Extraction and Isolation. The whole culture of *C. junghuhnii* (20 l) was filtered, and the filtrate was extracted four times with AcOEt. The org. layer was concentrated *in vacuo* to give a crude extract (40 g),

and the residue was subjected to CC (SiO_2; $CHCl_3$/MeOH gradient system) to give ten fractions. The fraction (665 mg) eluted with $CHCl_3$/MeOH (95:5, *v*/*v*) was subjected to repeated CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to produce three subfractions *Fr. 1* (259 mg), *Fr. 2* (165 mg), and *Fr. 3* (15 mg). *Fr. 2* was further purified by CC (SiO_2; petroleum ether/AcOEt 4:1) and (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1) to afford pure compound **1** (10 mg).

2,3,6-Trihydroxycaryol-5-en-7-one (=*(1*R,*2*R,*5*R,*6*R,*7*S,*8*R*)-6,7,10-Trihydroxy-4,4,8-trimethyltricyclo[6.3.1.0^{2,5}]dodec-9-en-11-one*; **1**). Colorless oil. R_f (PE/acetone 2:1): 0.60. $[\alpha]_D^{26.5} = -52.1$ ($c = 0.37$, $CHCl_3$). UV ($CHCl_3$): 277 (3.65). IR (KBr): 3422, 2953, 2933, 1734, 1667, 1459, 1406, 1367, 1222, 1175, 1070, 1059, 1024, 984, 936. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Table*. EI-MS: 266 (M^+), 248 ($[M - H_2O]^+$), 230 ($[M - 2\,H_2O]^+$). HR-ESI-MS: 289.1420 ($[M + Na]^+$, $C_{15}H_{22}NaO_4^+$; calc. 289.1415).

This project was supported by the *Chinese Academy of Sciences* (KSCX1-YW-R-24; KSCX2-YW-G-025) and the *National Basic Research Program of China* (2009CB522300).

REFERENCES

[1] X. L. Mao, 'The Macrofungi in China', Henan Science and Technology Press, Henan, 2000, p. 168.
[2] J. K. Liu, *Chem. Rev.* **2006**, *106*, 2209.
[3] J. K. Liu, *Chem. Rev.* **2005**, *105*, 2723.
[4] D.-Z. Liu, F. Wang, T.-G. Liao, J.-G. Tang, W. Steglich, H.-J. Zhu, J.-K. Liu, *Org. Lett*. **2006**, *8*, 5749.
[5] X.-D. Qin, Z.-J. Dong, J.-K. Liu, L.-M. Yang, R.-R. Wang, Y.-T. Zheng, Y. Lu, Y.-S. Wu, Q.-T. Zheng, *Helv. Chim. Acta* **2006**, *89*, 127.
[6] D.-Q. Luo, H.-J. Shao, H.-J. Zhu, J.-K. Liu, *Z. Naturforsch., C* **2005**, *60*, 50.
[7] C. J. Barrow, J. W. Blunt, M. H. G. Munro, *Aust. J. Chem*. **1988**, *41*, 1755.

Received July 29, 2008