Highly Acetylated Lanostane-Type Triterpenoids from *Coprinus cinereus* 120

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Two new highly acetylated lanostane-type triterpenoids, coprinacin A (**1**) and coprinacin B (**2**), were isolated from the basidiomycetous strain *Coprinus cinereus* 120. The chemical structures of **1** and **2** were elucidated by spectroscopic analyses, including 1D- and 2D-NMR experiments and FT-ICR-MS, and their *in vitro* cytotoxic activities against the human-tumor cell line HeLa were established as modest.

Introduction. – Terrestrial fungi continue to be an increasing source of potentially useful substances with a wide range of biological activities [1]. Recently, many terpenoids have been isolated from the genus *Coprinus*, such as illudins from *C. episcopalis*, heptemerons A – G from *C. heptemerus*, and coprinastatin from *C. cinereus* [2 – 4]. To explore more bioactive terpenoids from *Coprinus* sp., we focused our interest on the antitumor constituents of the leaf-litter inky cap fungus *Coprinus cinereus* 120, collected from the Kunming Institute of Botany, Yunnan, China. Here, we report the isolation, structure elucidation, and cytotoxic activity of two novel triterpenes, *i.e.*, coprinacin A (**1**) and coprinacin B (**2**) from *Coprinus cinereus* 120 (*Fig.*).

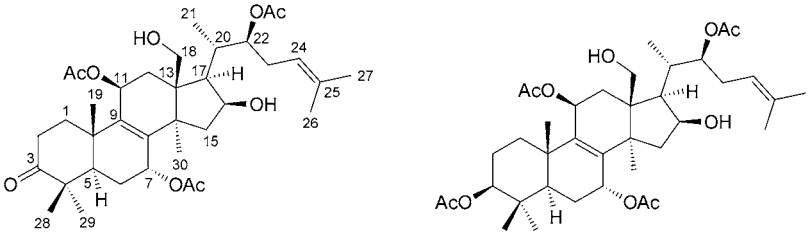

Figure. *Compounds* **1** *and* **2**, *isolated from* Coprinus cinereus *120*

Results and Discussion. – The strain *Coprinus cinereus* 120 was incubated for 16 d at 28° on potato-dexrose-agar (PDA) medium. The fermentation culture was extracted with AcOEt/MeOH/AcOH 16 : 3 : 1, and the extract partitioned between H_2O/AcOEt

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1:1. The AcOEt extract was chromatographed repeatedly by means of various columns (*RP-18*, *Sephadex LH-20*, and SiO_2) to yield compounds **1** and **2**.

Compound **1** was obtained as a white powder. Its molecular formula was determined to be $C_{36}H_{54}O_9$ according to the FT-ICR-MS data (*m/z* 653.3665 ($[M+Na]^+$)). The IR absorptions at 3444, 1731, and 1710 cm^{-1} indicated the presence of OH and C=O groups, respectively. The ^{13}C-NMR and DEPT spectra of **1** (*Table*) exhibited 36 signals: ten Me groups, seven CH_2 groups, of which one was O-bearing, eight CH groups, of which four were O-bearing, and eleven quaternary C-atoms, including four C=O groups. The structure of compound **1** was elucidated on the basis of the following evidence. Its ^{1}H-NMR spectrum (*Table*) showed nine *s* including three acetate Me groups at δ(H) 2.04, 2.06, and 2.11. The remaining six *s* at δ(H) 1.33, 1.63, 1.69, 1.09, 1.06, and 0.94 were assigned to Me(19), Me(26), Me(27), Me(28), Me(29), and Me(30), respectively. A *d* at δ(H) 0.97 ($J = 6.7$ Hz) was assigned to Me(21). Six characteristic downfield signals at δ(H) 5.56 (*d*, $J = 7.8$ Hz), 5.54 (*d*, $J = 8.0$ Hz), 5.28 (*t*, $J = 7.2$ Hz), 4.63 (br. *t*, $J = 4.6$ Hz), and 3.83 (*d*, $J = 11.5$ Hz) and 3.55 (*d*, $J = 12.2$ Hz) were due to H-atoms at O-bearing C-atoms and were assigned to H–C(7), H–C(11), H–C(22), H–C(16), and CH_2(18), respectively. The olefinic H-atom at δ(H) 5.08 (br. *t*, $J = 7.2$ Hz) indicated the presence of a lanostane-type side chain. The ^{13}C-NMR and DEPT spectra of compound **1** showed resonances for 36 C-atoms. The downfield resonances at δ(C) 216.1, 171.1, 170.2, and 169.8 were assigned to C(3) and the three ester C=O groups. Four CH and one CH_2 signals at δ(C) 69.4, 68.7, 75.5, 72.2 and 63.3 were assigned to the O-bearing atoms C(7), C(11), C(22), C(16), and C(18). The resonances at δ(C) 19.3, 17.9, 25.8, 21.5, 26.3, and 29.4 were ascribed to the Me groups (Me(19), Me(26), Me(27), Me(28), Me(29), and Me(30), resp.). The overall NMR data were in agreement with the known compound lanosta-8,24-dien-3-one [5]. Unambiguous assignments of the ^{1}H- and ^{13}C-NMR data were achieved by a combination of DEPT, HSQC, and HMBC results. From the analysis mentioned above, the chemical structure of coprinacin A (**1**) was established as (7α,11β,16β,22*S*)-7,11,16,18,22-pentahydroxylanosta-8,24-dien-3-one 7,11,22-triacetate.

Compound **2** was obtained as a white powder, and its molecular formula was determined as $C_{38}H_{58}O_{10}$ by FT-ICR-MS (*m/z* 697.3922 ($[M+Na]^+$)). The structure of **2** was elucidated to be a 3-*O*-acetylated derivative of **1** by comparison of its NMR spectra with those of **1** (*Table*). In **1**, C(3) (δ(C) 216.1) was a ketone group, whereas, in **2**, C(3) was a AcO-substituted CH group, with δ(C) 76.9 and δ(H) 4.68. Therefore, the chemical structure of coprinacin B (**2**) was identified as (3β,7α,11β,16β,22*S*)-lanosta-8,24-diene-3,7,11,16,18,22-hexol 3,7,11,22-tetraacetate.

The cytotoxic activities of **1** and **2** were tested by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method [6]. The IC_{50} values of **1** and **2** against the HeLa cell line were determined to be 34.6 μM and 41.0 μM, respectively.

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

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	2.56–2.62, 1.77–1.79 (2*m*)	34.5 (*t*)	1.56–1.59, 1.31–1.33 (2*m*)	29.6 (*t*)
CH_2(2)	2.43–2.47, 1.82–1.84 (2*m*)	34.1 (*t*)	1.90–1.96 (*m*), 1.62–1.65 (*m*, overlapped)	26.2 (*t*)
C(3) or H–C(3)	–	216.1 (*s*)	4.68 (*dd*, *J* = 11.2, 4.8)	76.9 (*d*)
C(4)	–	46.8 (*s*)		36.3 (*s*)
H–C(5)	1.82–1.84 (*m*)	47.5 (*d*)	1.62–1.65 (*m*, overlapped)	42.4 (*d*)
CH_2(6)	2.01 (*dd*, *J* = 13.5, 4.8), 1.73 (*d*, *J* = 13.8)	27.0 (*t*)	1.87–1.93 (*m*), 1.66–1.71 (*m*, overlapped)	23.0 (*t*)
H–C(7)	5.56 (*d*, *J* = 7.8)	69.4 (*d*)	5.50 (*d*, *J* = 7.4)	69.8 (*d*)
C(8)	–	138.2 (*s*)		137.6 (*s*)
C(9)	–	140.1 (*s*)		141.7 (*s*)
C(10)	–	38.1 (*s*)		38.2 (*s*)
H–C(11)	5.54 (*d*, *J* = 8.0)	68.7 (*d*)	5.56 (*d*, *J* = 7.8)	68.8 (*d*)
CH_2(12)	2.42–2.46 (*m*), 1.42 (*d*, *J* = 15.5)	39.7 (*t*)	2.38–2.42 (*m*), 1.47 (*d*, *J* = 15.5)	39.4 (*t*)
C(13)	–	47.8[a]) (*s*)		47.8[a]) (*s*)
C(14)	–	47.9[a]) (*s*)		47.9[a]) (*s*)
CH_2(15)	2.07–2.09, 1.82–1.84 (2*m*)	43.0 (*t*)	2.01 (*dd*, *J* = 13.5, 3.1), 1.80–1.83 (*m*)	43.0 (*t*)
H–C(16)	4.63 (br. *t*, *J* = 4.6)	72.2 (*d*)	4.62 (*br. t*, *J* = 4.5)	72.3 (*d*)
H–C(17)	1.82–1.84 (*m*)	49.7 (*d*)	1.83–1.84 (*m*)	49.7 (*d*)
CH_2(18)	3.83 (*d*, *J* = 11.5), 3.55 (*d*, *J* = 12.2)	63.3 (*t*)	3.90 (*d*, *J* = 11.5), 3.55 (*d*, *J* = 12.0)	63.3 (*t*)
Me(19)	1.33 (*s*)	19.3 (*q*)	1.22 (*s*)	19.8 (*q*)
H–C(20)	2.13–2.16 (*m*)	33.4 (*d*)	2.12–2.14 (*m*)	33.5 (*d*)
Me(21)	0.97 (*d*, *J* = 6.7)	12.6 (*q*)	0.98 (*d*, *J* = 6.6)	12.7 (*q*)
H–C(22)	5.28 (*t*, *J* = 7.2)	75.5 (*d*)	5.30 (*t*, *J* = 7.2)	75.5 (*d*)
CH_2(23)	2.34–2.38, 2.13–2.16 (2*m*)	31.2 (*t*)	2.34–2.38, 2.15–2.18 (2*m*)	31.2 (*t*)
H–C(24)	5.08 (br. *t*, *J* = 7.2)	119.5 (*d*)	5.10 (br. *t*, *J* = 7.2)	119.6 (*d*)
C(25)	–	134.3 (*s*)	–	134.2 (*s*)
Me(26)	1.63 (*s*)	17.9 (*q*)	1.63 (*s*)	18.0 (*q*)
Me(27)	1.69 (*s*)	25.8 (*q*)	1.69 (*s*)	25.8 (*q*)
Me(28)	1.09 (*s*)	21.5 (*q*)	0.94 (*s*)	22.0 (*q*)
Me(29)	1.06 (*s*)	26.3 (*q*)	0.82 (*s*)	27.4 (*q*)
Me(30)	0.94 (*s*)	29.4 (*q*)	0.99 (*s*)	29.7 (*q*)
*Ac*O–C(22)	2.04 (*s*)	171.1 (*s*), 21.4 (*q*)	2.05 (*s*)	171.1 (*s*), 21.4 (*q*)
*Ac*O–C(7)	2.06 (*s*)	170.2 (*s*), 21.2 (*q*)	2.07 (*s*)	170.2 (*s*), 21.2[a]) (*q*)
*Ac*O–C(11)	2.11 (*s*)	169.8 (*s*), 21.8 (*q*)	2.08 (*s*)	169.9 (*s*), 21.8[a]) (*q*)
*Ac*O–C(3)	–	–	2.09 (*s*)	170.6 (*s*), 21.5[a]) (*q*)

[a]) Assignments may be interchanged.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 and 80–100 mesh; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China), silica gel GF_{254} (*Merck*), *RP-18* (*Merck*), and *Sephadex LH-20* (*Amersham Biosciences*). TLC: precoated silica gel GF_{254} plates (0.20–0.25 mm, *Qingdao Marine Chemical Factory*). Optical rotations: *Perkin–Elmer 341* automatic polarimeter; in MeOH. IR Spectra: *Nicolet-Avatar-330* FT-IR spectrometer; in KBr; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker DRX-600* spectrometer, at 600 (^1H) and 150 MHz (^{13}C) in $CDCl_3$; δ in ppm rel. to Me_4Si as internal standard, J in Hz. FT-ICR-MS: *Bruker Apex III*; in *m/z*.

Isolation and Fermentation of the Fungal Strain. The fungus was collected in the Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China. Both a traditional morphology and an internal transcribed spaces (ITS) sequencing were performed to characterize it as *Coprinus cinereus.* Fermentation was performed for 16 d at 28° on potato-dextrose-agar (PDA) media (10 l).

Extraction and Isolation. The cultured agar was chopped, diced, and extracted three times with AcOEt/MeOH/AcOH 16:3:1 at r.t. overnight. The org. soln. was collected by filtration. The combined filtrates were concentrated to remove the org. solvents. The aq. soln. was extracted with AcOEt. The combined orgAcOEt layer, upon solvent removal, yielded the AcOEt extract (4.7 g). The AcOEt extract (4.7 g) was subjected to MPLC (*RP-18* silica gel (130 g), H_2O, then 30, 50, 70, and 100% MeOH/H_2O, 2 l for each gradient step): *Fractions 1–5*. *Fr. 4* (680 mg) was separated by CC (*Sephadex LH-20* (140 g), MeOH): *Fr. 4a*. *Fr. 4a* (330 mg) was then subjected to MPLC/*RP-18* (80 g), MeOH/H_2O 68:32, 72:28, and 1:1): *Fr. 4a1* and *Fr. 4a2*. *Fr. 4a1* (30 mg) was further purified by CC (SiO_2, petroleum ether/AcOEt 4:1): **1** (22 mg). *Fr. 4a2* (5 mg) was finally purified by CC (SiO_2, petroleum ether/AcOEt 6:1): **2** (2.0 mg).

Coprinacin A (*=(7α,11β,16β,22*S*)-7,11,16,18,22-Pentahydroxylanosta-8,11-dien-3-one 7,11,22-Triacetate*; **1**): White powder. $[\alpha]_D^{20} = +18.9$ ($c = 0.18$, MeOH). IR (KBr): 3442, 2972, 1731, 1710, 1442, 1372, 1231, 1019. ^1H- and ^{13}C-NMR: *Table.* FT-ICR-MS: 653.3665 ($[M+Na]^+$; calc. 653.3660).

Coprinacin B (*=(3β,7α,11β,16β,22*S*)-Lanosta-8,24-diene-3,7,11,16,18,22-hexol 3,7,11,22-Tetraacetate*; **2**). White powder. $[\alpha]_D^{20} = +36.1$ ($c = 0.18$, MeOH). IR (KBr): 3444, 2967, 1726, 1727,1442, 1372, 1240,1020. ^1H- and ^{13}C-NMR: *Table.* FT-ICR-MS: 697.3922 ($[M+Na]^+$; calc. 697.3922).

Biological Assay. The cytotoxicity of **1** and **2** was investigated with the human-cancer cell line HeLa, following the MTT standards, and cisplatin was used as a positive control in this experiment.

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