Illudins C_2 and C_3, New Illudin C Derivatives from *Coprinus atramentarius* ASI20013

IN-KYOUNG LEE, CHEOL-YOUN JEONG, SOO-MUK CHO, BONG-SIK YUN, YANG-SUP KIM†, SEUNG-HUN YU††, HIROYUKI KOSHINO††† and ICK-DONG YOO*

Korea Research Institute of Bioscience and Biotechnology, KIST, P. O. Box 115, Yusong, Taejon 305-600, Korea
†Applied Microbiology Division, National Agricultural Science & Technology Institute, RDA, Suwon, 440-707, Korea
††Department of Agricultural Biology, Chungnam Nat'l University, Taejon, 305-764, Korea
†††The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako, Saitama 351-01, Japan

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In the course of screening for antimicrobial antibiotics against *Staphylococcus aureus* from basidiomycetes, we isolated two active compounds, illudins C_2 (**1**) and C_3 (**2**), from the culture broth of *Coprinus atramentarius*, which was well known to produce coprine with aldehyde dehydrogenase inhibitory activity[1~3]. This paper presents the fermentation, isolation, structural determination and antimicrobial activities of **1** and **2**.

The strain ASI20013 grown on potato sucrose agar medium was used to inoculate six 500-ml Erlenmeyer flasks containing 100 ml of the seed medium consisting of yeast extract 0.4%, malt extract 1.0% and glucose 0.4% (pH 6.0 before sterilization). The flasks were shaken on a rotary shaker for three days at 27°C. The seed culture was transferred into a 50-liter jar fermenter containing 30 liters of the above medium for production of compounds **1** and **2**, and cultivation was carried out at 28°C for three days with aeration of 3 liters/minute and agitation of 130 rpm.

Compounds **1** and **2** were isolated from the fermentation broth (30 liters) by monitoring the antimicrobial activity against *S. aureus*. The fermentation broth was separated into filtrate and mycelia by centrifugation. The broth filtrate was applied to a column of Diaion HP-20. The column was washed with 30% MeOH and eluted with 70% MeOH. The active eluate was concentrated *in vacuo* and the resulting aqueous solution was extracted with ethyl acetate. After concentration of the solvent layer *in vacuo*, the residue was chromatographed on a silica gel column with $CHCl_3$-MeOH (10:1) as an eluent. The concentrate of the active eluate was applied to ODS column and eluted with 40% MeOH to give two active fractions. Each of two active fractions was further purified by preparative TLC (silica gel TLC, Merck 60 F_{254}), which was developed with $CHCl_3$-MeOH (10:1). Two active compounds with Rf values of 0.5 and 0.4 for illudins C_2 and C_3, respectively, were scraped off the TLC plates and extracted with $CHCl_3$-MeOH (3:1) to give 5 mg of **1** and 3 mg of **2**.

The physico-chemical properties of **1** and **2** are summarized in Table 1. The EI mass spectroscopies of both compounds gave a peak at *m/z* 248 (M^+) and fragments at *m/z* 233 ($M-CH_3$), 220 ($M-CO$), 215, 187 and 159.

Table 1. Physico-chemical properties of **1** and **2**.

	1	2
Appearance	Oil	Oil
Molecular formula	$C_{15}H_{20}O_3$	$C_{15}H_{20}O_3$
HREI-MS (*m/z*)		
found	248.1419 (M^+)	248.1434 (M^+)
calcd.	248.1412	248.1413
$[\alpha]_D^{20}$	+20.0° (*c* 0.30, MeOH)	-15.4° (*c* 0.26, MeOH)
UV λ_{max}^{MeOH} nm (ε)	205 (9700) 264 (5800)	205 (11500) 270 (7300)
IR ν_{max} (KBr) cm^{-1}	3380, 1660, 1600, 1040	3380, 1660, 1600, 1040

Table 2. The ^{13}C NMR chemical shifts (ppm) of **1**, **2** and illudin C.

Carbon No.	1	2	Illudin C[a]
1	188.2	188.1	185.8
2	149.3	148.9	149.6
2-CH_2	116.9	116.9	115.5
3	33.9	33.7	34.0
4	70.7	70.5	70.2
4-CH_3	25.1	24.9	26.4
4a	173.1	173.1	171.2
5	40.3	40.2	48.4
6	43.4	42.9	38.1
6-CH_3	25.7	25.8	29.6
			29.7
6-CH_2OH	70.3	70.1	
7	43.9	43.5	45.2
7a	135.6	135.3	135.6
8	5.2	5.2	5.0
9	13.4	13.2	13.6

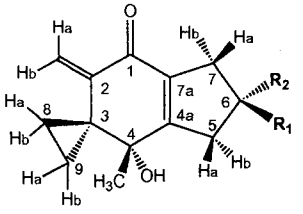
Compounds **1** and **2** were measured in CD_3OD and illudin C was measured in acetone-d_6.
[a] Data taken from Ref. 1.

Table 3. The ^1H NMR chemical shifts (ppm) of **1**, **2** and illudin C.

Positions	1	2	Illudin C[a]
2-CH_2 a	5.87 (s)	5.82 (s)	5.82
b	5.19 (s)	5.15 (s)	5.14
4-CH_3	1.34 (s)	1.28 (s)	1.34
5a	2.88 (d, 19.0)[b]	2.69 (d, 19.0)	2.67
b	2.38 (d, 19.0)	2.49 (d, 19.0)	2.50
6-CH_3	1.11 (s)	1.10 (s)	1.10, 1.14
6-CH_2OH	3.43 (s)	3.31 (s)	
7a	2.60 (d, 16.0)	2.54 (d, 16.0)	2.39[c]
b	2.28 (d, 16.0)	2.22 (d, 16.0)	2.37[c]
8a	1.03 (m)	0.99 (m)	1.05
b	0.87 (m)	0.82 (m)	0.88
9a	0.15 (m)	0.13 (m)	0.12
b	1.13 (m)	1.08 (m)	1.18

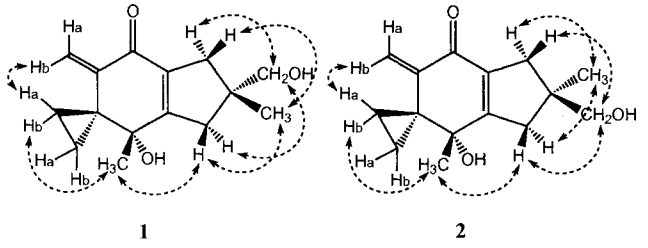
Compounds **1** and **2** were measured in CD_3OD and illudin C was measured in acetone-d_6.
[a] Data taken from Ref. 1.
[b] Proton resonance multiplicity and coupling constant (Hz) in parentheses.
[c] Assignments interchangeable.

Fig. 1. The structures of illudins C_2 (**1**), C_3 (**2**) and C.



1 : $R_1 = -CH_3$, $R_2 = -CH_2-OH$
2 : $R_1 = -CH_2-OH$, $R_2 = -CH_3$
Illudin C : $R_1 = R_2 = -CH_3$

Fig. 2. The relative stereochemistries of illudins C_2 (**1**) and C_3 (**2**) elucidated by NOESY data.



1 **2**

The arrows indicate NOE correlations.

Compounds **1** and **2** isolated as oil were determined to have a molecular formula of $C_{15}H_{20}O_3$ by high-resolution EI mass spectroscopic analysis and by ^1H and ^{13}C NMR spectroscopic analyses. The UV spectrum of **1** also was similar to that of **2**. The IR absorptions of both compounds at 3380 and 1660 cm^{-1} suggested the presence of hydroxyl and carbonyl groups in their structures. All of these evidences including ^1H and ^{13}C NMR spectra suggested that **1** may be a stereo isomer of **2**.

The ^1H and ^{13}C NMR spectral data of **1** and **2** are summarized in Table **2** and **3**. The ^1H and ^{13}C NMR spectra of **1** and **2** are quite similar to those of illudin C[4,5] as shown in Tables 2 and 3. The structures of compounds **1** and **2** were assigned by a direct comparison with that of illudin C[4]. In ^1H and ^{13}C NMR spectra of compounds **1** and **2**, an oxylated methylene signal at 3.43 (^1H) and 70.3 (^{13}C) ppm for **1**, and at 3.31 (^1H) and 70.1 (^{13}C) ppm for **2** replaced a methyl signal (6-CH_3) in illudin C (Tables 2 and 3). Thus plenary structures of **1** and **2** were determined as shown in Fig. 1. As the forgoing, Compounds **1** and **2** were suggested to be stereo isomer with $[\alpha]_D$ values at +20 and −15, respectively. The NOE experiments of **1** and **2** were consistent with the diastereoisomeric relationship between **1** and **2**, permitting us to assign the relative configuration at C-4 and C-6. The NOESY data of **1** showed the NOEs between 4-CH_3 and 5-Ha, and 5-Ha and 6-CH_3 while the NOEs between 4-CH_3 and 5-Ha, and 5-Ha and 6-CH_2OH were observed in **2**, as shown in Fig. 2. This result revealed that 4-CH_3 and 6-CH_3 situated on the same plane in **1** were on anti-plane in **2**. Their absolute stereochemistries were not determined.

Table 4. Antimicrobial activity of **1** and **2** in the serial dilution assay.

Test organisms	MIC (μg/ml)[a]	
	1	**2**
Bacteria		
Staphylococcus aureus 209	12	12
S. aureus R209	25	25
Escherichia coli	2	1
Salmonella typhymurium	>100	>100
Fungi		
Botrytis cinerea	25	25
Rhizoctonia solani	>100	>100
Phytophthora capsici	25	25
Fusarium oxysporum	>100	>100
Pyricularia oryzae	>100	>100

[a] Determined after 24 hours of incubation at 37°C for the bacteria and after 48 hours of incubation at 28°C for the fungi.

Antimicrobial activity against some bacteria and fungi for **1** and **2** was evaluated in agar dilution method as shown in Table 4. The both compounds showed same minimum inhibitory concentrations of 12 and 25 μg/ml against *Staphylococcus aureus* 209 and *S. aureus* R209, multi-drug resistant strain, respectively.

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