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NOTES

Quinolactacins A1 and A2, New Acetylcholinesterase Inhibitors from *Penicillium citrinum*

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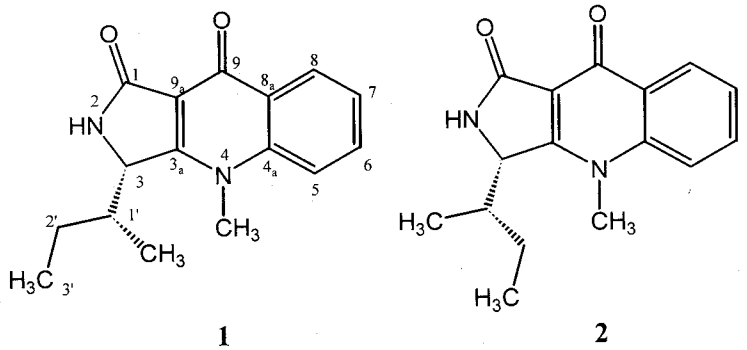
Alzheimer disease is a neurodegenerative disorder that is the most common cause of dementia among the elderly. The neurophathological evidences have demonstrated that cholinergic functions declined in the basal forebrain and cortex in senile dementia of the Alzheimer type[1,2]. Accordingly, enhancement of cholinergic neurotransmission have been considered as one potential therapeutic approach against Alzheimer disease. One treatment strategy to enhance cholinergic functions is the use of acetylcholinesterase (AChE, EC 3.1.1.7) inhibitors to increase the amount of acetylcholine present in the synapses between cholinergic neurons[3,4]. Acetylcholinesterase inhibitors like tacrine, one of the most extensively evaluated acetylcholinesterase inhibitors, have been shown to significantly improve cognitive function in Alzheimer disease[5,6]. Tacrine, however, has been known to cause hepatotoxic side effects by also inhibiting butyrylcholinesterase (BuChE, EC 3.1.1.8) which is found in plasma[7]. In this respect, an inhibitor selective for acetylcholinesterase has attracted particular attention for treatment of the Alzheimer-type dementia. Arisugacins A~H with a meroterpenoid structure have been isolated as selective acetylcholinesterase inhibitors of microbial origin[8~12]. In the course of our screening for selective acetylcholinesterase inhibitors from microbial metabolites, we isolated two diastereomeric compounds named quinolactacin A1 (**1**) and quinolactacin A2 (**2**) from solid state fermentation of *Penicillium citrinum* 90648 (Fig. 1). **1** is a new diastereomer of **2**. **2** is assumed to have the same planar structure and relative configuration as quinolactacin A[13], a very recently reported compound, of which the stereochemistry has not been reported. We report here the isolation, physico-chemical properties, and structure determination of **1** and **2**.

The producing strain fb90648 was isolated from a soil sample collected in corn field around Sokcho-city, Kangwon-do, Korea and assigned to the *Penicillium citrinum* 90648. Fermentation was carried out in solid state because **1** and **2** were not produced in liquid culture media containing glucose 2%, yeast extract 0.2%, polypeptone 0.5%, $MgSO_4$ 0.05%, and KH_2PO_4 0.1% (pH 5.7 before sterilization). A piece of strain fb90648 from a mature plate culture was inoculated into a 500 ml Erlenmeyer flask containing 80 ml of steril seed liquid medium with the

Fig. 1. Relative structures of quinolactacin A1 (**1**) and quinolactacin A2 (**2**).



1 **2**

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

	1	**2**
Appearance	white powder	white powder
EI-MS (*m/z*)	270 $(M)^+$	270 $(M)^+$
HREI-MS (*m/z*)		
found.	270.1373	270.1361
calcd.	270.1368	270.1368
Molecular formula	$C_{16}H_{18}N_2O_2$	$C_{16}H_{18}N_2O_2$
UV λ_{max} nm (log ε)(MeOH)	215 (4.40), 247 (4.23), 256 (4.23) 315 (3.94), 327 (3.93)	215 (4.30), 248 (4.11), 256 (4.10), 315 (3.83), 327 (3.78)
IR (KBr) γcm^{-1}	3425, 2923, 2854, 1698, 1610, 1525, 1462, 1125	3422, 2923, 2854, 1700, 1609, 1525, 1462, 1125
CD λ_{ext} nm (Δε)(MeOH)	328 (-956), 305 (750), 254 (-1272), 230 (4821), 208 (-1675)	327 (859), 305 (-175), 254 (926), 229 (-3523), 208 (167)
HPLC $(R_t)^a$ (minute)	9.7	10.6

[a]Column, Chirex (R)-NGLY and DNB (4 x 250 mm); solvent, MeOH-H_2O (45:50); flow rate, 1 ml/min; UV absorbance at 315 nm.

above composition and cultured on a rotary shaker (150 rpm) at 28°C for 3 days. For the production of **1** and **2**, 5 ml of the seed culture was transferred into one-liter Erlenmeyer flasks (48 flasks) containing moistured wheat bran which were cultivated for 7 days at 28°C under a stationary conditions. The solid culture was extracted with 80% aqueous acetone. After the acetone extracts were filtered and concentrated, the resulting aqueous solution was extracted with an equal volume of ethyl acetate three times and the ethyl acetate layer was concentrated *in vacuo*. The resultant residue was subjected to SiO_2 (Merck Art No. 7734.9025) column chromatography followed by elution with $CHCl_3$-MeOH (10:1). The active fractions were pooled and concentrated *in vacuo* to give an oily residue. The residue was applied again to a Sephadex LH-20 and then eluted with MeOH. The active fraction dissolved in MeOH was further purified by reverse phase HPLC column (20×250 mm, YMC C_{18}) chromatography with a photodiode array detector. The column was eluted with CH_3CN-H_2O (35:65) at a flow rate of 6 ml/minute to afford a mixture (90 mg) of two diastereomers (**1** and **2**) at a retention time of 11.5 minutes as a white powder. The diastereomers were separated by a Chiral HPLC column (Chirex (R)-NGLY and DNB 4.0×250 mm, Phenomenex). The elution with MeOH-H_2O (45:50) at a flow rate of 1.0 ml/minute afforded **1** with a retention time of 9.7 minutes and **2** at 10.6 minutes.

The physico-chemical properties of **1** and **2** are summarized in Table 1. **1** and **2** were determined to have the same molecular formula $C_{16}H_{18}N_2O_2$ on the basis of high resolution EI-MS [M^+, 270.1373 *m/z* (+0.5 mmu error) and 270.1361 *m/z* (−0.7 mmu error), respectively] in combination with ^1H and ^{13}C NMR data. The compounds both are soluble in methanol, dimethlyl sulfoxide and $CHCl_3$, and insoluble in water, ether and *n*-hexane. The UV absorption spectra of **1** and **2** showed almost the same absorption maxima at 213~214, 247, 256, 303, 314, and 327. Also, the IR spectra of **1** and **2** revealed almost the same absorption bands attributable to carbonyl groups (1698 and 1700 cm^{-1}, respectively) and NH groups (3425 and 3422 cm^{-1}, respectively). In contrast, the CD spectrum (λ_{ext} nm ($\Delta\varepsilon$) 328 (−956), 305 (750), 254 (−1272), 230 (4821), 208 (−1675) in MeOH) of **1** showed the opposite pattern to that (λ_{ext} nm ($\Delta\varepsilon$) 327 (859), 305 (−175), 254 (926), 229 (−3523), 208 (167) in MeOH) of **2**. This data suggested that **1** could be a stereomeric isomer of **2**.

The ^1H NMR and ^{13}C NMR spectral data (Table 2) of **1** with ^1H-^1H COSY, HMQC and DEPT spectral data revealed the presence of a 1, 2-disubstituted benzene ring, a CH_3–CH_2–$CH(CH_3)$–CH– group, two carbonyl carbons (δ 169.9 and δ 173.0), two sp^2 quaternary carbons (δ 110.3 and δ 163.9), an *N* or *O*-linked methyl (δ_H 3.80, 3H, s; δ_C

Table 2. ^{1}H and ^{13}C NMR data for **1** and **2**.

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		169.9 C		169.7 C
3	4.80 (1H, d, 2.5)[a]	57.9 CH	4.70 (1H, d, 2.6)	60.2 CH
3a		163.9 C		163.6 C
4-CH_3	3.80 (3H, s)	35.8 CH_3	3.82 (3H, s)	35.9 CH_3
4a		140.9 C		141.0 C
5	7.47 (1H, d, 8.2)	115.6 CH	7.51 (1H, d, 8.2)	115.7 CH
6	7.66 (1H, ddd, 8.2, 7.5, 1.4)	132.8 CH	7.62 (1H, ddd, 8.2, 7.5, 1.6)	132.8 CH
7	7.29 (1H, dd,7.6, 7.5)	124.7 CH	7.36 (1H, dd,7.5, 7.6)	124.7 CH
8	8.23 (1H, dd, 7.6, 1.4)	126.6 CH	8.30 (1H, dd, 7.6, 1.6)	126.6 CH
8a		127.8 C		127.8 C
9		173.0 C		172.9 C
9a		110.3 C		110.3 C
1'	2.13 (1H, m)	36.5 CH	2.17 (1H, m)	36.5 CH
1'-CH_3	0.51 (3H, d, 6.7)	11.4 CH_3	1.21 (3H, d, 6.9)	17.3 CH_3
2' H_a	1.62 (1H, m)	27.7 CH_2	H_a 0.98 (1H, m)	20.9 CH_2
H_b	1.48 (1H, m)		H_b 0.84 (1H, m)	
3'	1.09 (3H, t, 7.4)	11.7 CH_3	0.69 (3H, t, 7.4)	11.3 CH_3
NH	7.37 (1H, brs)		7.44 (1H, brs)	

All spectra of **1** and **2** were recorded at 600 MHz for ^{1}H and 150 MHz for ^{13}C in CD_3Cl.
[a]Proton resonance multiplicity and coupling constant (J = Hz) are in parenthesis.
The assignments were aided by ^{1}H-^{1}H COSY, DEPT, NOESY, HMQC, and HMBC.

35.8, q), and one exchangeable proton (δ 7.39). Taking into account the molecular formula and the *N* or *O*-linked methyl group, the exchangeable proton should be assigned to be an NH group. In the HMBC spectrum (Fig. 2), long range couplings were observed from the protons of N–CH_3 to the olefinic carbon (C-5) and the sp^2 quaternary carbon (C-4_a) of the benzene ring and the two sp^2 quaternary carbons at δ 110.3 (C-9_a) and δ 163.9 (C-3_a). Long range couplings were also observed from the olefinic proton (H-8) of the benzene ring to the carbonyl carbon at δ 173.0 (C-9). This HMBC data suggested the presence of a quinolone moiety. On the other hand, the NH proton at δ 7.39 was long-range coupled to the two sp^2 quaternary carbons (C-3_a and C-9_a). In ^{1}H-^{1}H COSY spectrum (Fig. 2) in DMSO-d_6, the NH proton was also coupled to the methine proton (H-3) of $^{3'}CH_3$–$^{2'}CH_2$–$^{1'}CH(CH_3)$–^{3}CH– group which was, in turn, long-range coupled to the other carbonyl carbon at δ 169.9 (C-1) and the two sp^2 quaternary carbons (C-3_a and C-9_a). These spectral data indicated that a γ-lactam ring containing a *sec*-butyl group at C-3 is present in its structure and the γ-lactam ring should be conjugated at C-3_a and C-9_a of the quinolone moiety. The conjugated pattern of the γ-lactam ring was determined by the NOEs observed

Fig. 2. ^{1}H-^{1}H COSY and HMBC data of **1**.

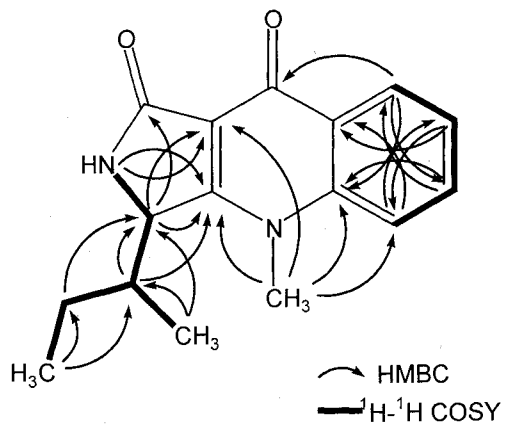
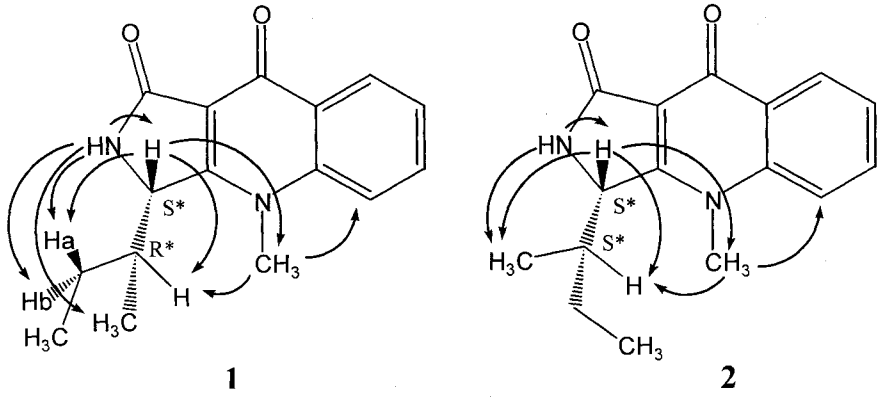


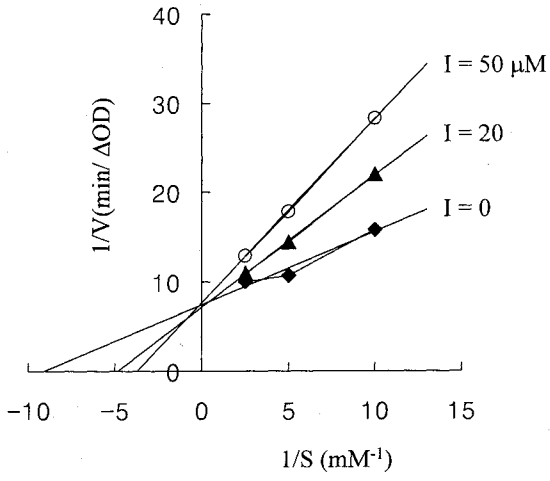
Fig. 3. NOE data of **1** and **2**.



from the protons of N–CH_3 to the protons of H-5, H-3, and H-1′ (Fig. 3). Thus the planar structure of **1** was elucidated. The relative stereochemistry of **1** was examined by NOESY spectrum in DMSO-d_6 (Fig. 3). NOEs were observed between H-3 and both of H-1′ and H_a-2′ while no NOEs was observed between H-3 and CH_3-1′. NOEs were also observed between the NH and H-3, H_2-2′, and CH_3-1′. These spectral data indicated that the relative stereochemistries of C-3 and C-1′ were *S** and *R**, respectively, with anti configuration of H-3 and CH_3-1′. This configuration was also supported by the high field-shifted chemical shift (δ_H 0.51, δ_C11.4) of CH_3-1′ which could be induced by anisotrophic effect of the pyrroloquinolone ring. Thus the structure of **1** was determined as shown in Fig. 1.

The ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1** (Table 2). The only difference between **1** and **2** in ^1H and ^{13}C NMR data with HMQC data was that the methylene of H_2-2′ and the methyl of H_3-3′ was upshield-shifted in **2** while the methyl of CH_3-1′ was downfield-shifted. The ^1H NMR spectral data with ^1H-^1H COSY also revealed the presence of CH_3–CH_2–$CH(CH_3)$–CH– group like **1**. The HMBC spectral data (data not shown) confirmed that the planar structure of **1** was the same as that of **2**. Together with CD spectral data, these NMR spectral data indicated that **2** could be a diastereoisomer of **1**. The relative stereochemistry of **2** was examined by NOESY spectrum in DMSO-d_6 (Fig. 3). NOEs were observed between H-3 and CH_3-1′ while no NOEs was observed between H-3 and both of H-1′ and H_2-2′. NOEs were also observed between the proton of NH and H-3 and CH_3-1′. These data indicated that the relative

Fig. 4. Lineweaver-Burk plot of inhibition of acetylcholinesterase by **2**.



stereochemistries of C-3 and C-1′ were *S** and *S**, respectively, with anti configuration of H-3 and H_2-2′. Thus, **2** was determined to be a diastereomer of **1** as shown in Fig. 1.

1 and **2** are closely related to each other, differing only by stereochemistry at C-1′. **1** and **2** have a very unique pyrrolo[3,4-*b*]quinolone skeleton of microbial origin. These compounds have the same planar structure as quinolactacin A which was reported very recently as the inhibitor of tumor necrosis factor production induced by murine peritoneal macrophage[13]. The stereochemistry of quinolactacin A has not been reported. Since the chemical shifts of ^1H and ^{13}C NMR of **2** rather than **1** was very

similar to those of quinolactacin A[14], the relative configuration of **2** is assumed to be the same as that of quinolactacin A. Anti-acetylcholinesterase activity of **1** and **2** was evaluated in Ellman's coupled acetylcholinesterse assay[15]. Interestingly, **2** showed 14-times higher inhibitory activity against acetylcholinesterase than **1**. **2** inhibited acetylcholinesterase in a dose-dependent fashion with an IC_{50} (μM) value of 19.8 while **1** showed the weak inhibitory activity with an IC_{50} (μM) value of 280. The inhibition of **2** was competitive with substrate (Fig. 4). The *Ki* and *Km* values for acetylcholinesterase were 2.39×10^{-5} M and 1.09×10^{-4} M, respectively. **2** exhibited the inhibitory activity selective for acetylcholinesterase with anti-butyrylcholinesterase activity of an IC_{50} (μM) value of 650. As a positive control, tacrine had a very low selectivity with a stronger inhibitory activity on butyrylcholinesterase (IC_{50} (μM); 0.006) rather than acetylcholinesterase (IC_{50} (μM); 0.12) in this assay system.

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