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Quinolactacins A, B and C: Novel Quinolone Compounds from *Penicillium* sp. EPF-6

II. Physico-chemical Properties and Structure Elucidation

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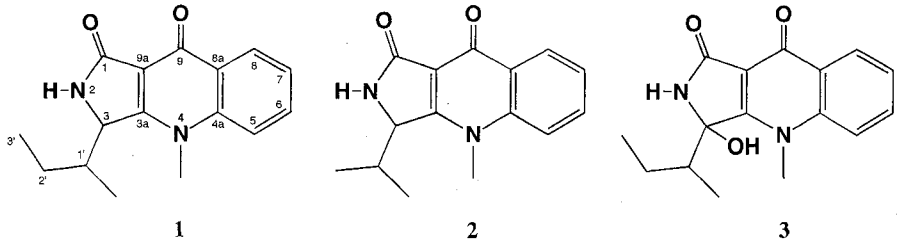
Three novel quinolone compounds, quinolactacins A (**1**), B (**2**) and C (**3**), have been found from the fermentation broth of *Penicillium* sp. EPF-6, a fungus isolated from the larvae of mulberry pyralid (*Margaronia* pyloalis Welker). The molecular formulas of **1**, **2** and **3** were determined to be $C_{16}H_{18}N_2O_2$, $C_{15}H_{16}N_2O_2$ and $C_{16}H_{18}N_2O_3$, respectively by FAB-MS and NMR spectral analyses. The structures of these compounds have a novel quinolone skeleton with a γ-lactam ring consisting of $C_{12}H_8N_2O_2$ as the common chromophore.

As a promising source of biologically active compounds, we are focusing on entomophathogenic fungi and their secondary metabolites. We have reported two novel protein kinase inhibitors, pyridovericin and pyridomacrolidin, which were isolated from the entomopathogenic fungus, *Beauveria bassiana* EPF-5[1,2]. In the course of our continuing HPLC screening program for new bioactive compounds, we discovered three novel quinolone skeleton compounds designated as quinolactacins A (**1**), B (**2**) and C (**3**) (Fig. 1), from the fermentation broth of *Penicillium* sp. EPF-6 isolated from the larvae of mulberry pyralid (*Margaronia* pyloalis Welker). In the previous paper, we described the screening, the isolation procedure and the biological properties of these quinolactacins[3]. The

Fig. 1. Structures of quinolactacins A (**1**), B (**2**) and C (**3**).



1 **2** **3**

Table 1. Physico-chemical properties of quinolactacins A (**1**), B (**2**) and C (**3**).

	1	**2**	**3**
Appearance	White powder	White powder	White powder
MP	262~265°C (dec.)	260~263°C (dec.)	180~185°C (dec.)
$[\alpha]_D^{25}$ (DMSO)	+17.9° (*c* 0.13)	-3.3° (*c* 0.15)	+5.9° (*c* 0.19)
Molecular formula	$C_{16}H_{18}N_2O_2$	$C_{15}H_{16}N_2O_2$	$C_{16}H_{18}N_2O_3$
Molecular weight	270	256	286
FAB-MS (*m/z*)	271 $(M+H)^+$, 269 $(M-H)^-$	257 $(M+H)^+$	287 $(M+H)^+$
HRFAB-MS (*m/z*)			
Found :	271.1439 $(M+H)^+$	257.1291 $(M+H)^+$	287.1388 $(M+H)^+$
Calcd. :	271.1446 for $C_{16}H_{19}N_2O_2$	257.1290 for $C_{15}H_{17}N_2O_2$	287.1395 for $C_{16}H_{19}N_2O_3$
UV λ_{max}^{MeOH} nm (ε)	215 (31,300), 248 (20,150), 256 (20,250), 315 (11,150), 327 (10,150)	214 (24,800), 248 (15,650), 255 (15,600), 314 (8,500), 327 (7,850)	215 (31,050), 250 (18,900), 258 (17,850), 315 (12,200), 328 (11,750)
IR ν_{max}^{KBr} (cm^{-1})	3293, 2963, 2934, 1702, 1605, 1547, 1524, 1464, 1422, 1269, 1221	3265, 2965, 2930, 1690, 1605, 1545, 1525, 1464, 1421, 1268, 1223	3230, 2967, 2933, 1704, 1606, 1545, 1520, 1463, 1420, 1267,
TLC (Rf value)[a]	0.73	0.82	0.27

[a] Silica gel TLC (Merck No. 5715) : $CHCl_3$-MeOH (4 : 1)

compound **1** showed inhibitory activity against tumor necrosis factor (TNF) production by murine macrophages and macrophage-like J774.1 cells stimulated with lipopolysaccharide (LPS)[4,5]. The structures of **1**, **2** and **3** are unique in that a quinolone skeleton is conjugated with a γ-lactam ring. In this paper, we describe the physico-chemical properties and structural elucidation of **1**, **2** and **3**.

Results and Discussion

Physico-chemical Properties

The physico-chemical properties of quinolactacins (**1**~**3**) are summarized in Table 1. Compounds **1**, **2** and **3** were obtained as white powders. The molecular formulas of **1**, **2** and **3** were established as $C_{16}H_{18}N_2O_2$ [found *m/z* 271.1439 $(M+H)^+$, calcd. 271.1446 for $C_{16}H_{19}N_2O_2$], $C_{15}H_{16}N_2O_2$ [found *m/z* 257.1291 $(M+H)^+$, calcd. 257.1290 for $C_{15}H_{17}N_2O_2$] and $C_{16}H_{18}N_2O_3$ [found *m/z* 287.1388 $(M+H)^+$, calcd. 287.1395 for $C_{16}H_{19}N_2O_3$], respectively, on the basis of high-resolution FAB-MS and NMR spectral analyses. Three compounds showed similar UV absorption maxima in MeOH [**1**: 215 (ε 31,300), 248 (ε 20,150), 256 (ε 20,250), 315 (ε 11,150) and 327 nm (ε 10,150), **2**: 214 (ε 24,800), 248 (ε 15,650), 255 (ε 15,600), 314 (ε 8,500) and 327 nm (ε 7,850), **3**: 215 (ε 31,050), 250 (ε 18,900), 258 (ε 17,850), 315 (ε 12,200) and 328 nm (ε 11,750)], suggesting the presence of the same chromophore. Each quinolactacin exhibited IR absorption (KBr) due to the NH groups (3293, 3265 and 3230 cm^{-1}) and carbonyl groups (1702, 1690 and 1704 cm^{-1}). These compounds were soluble in DMSO, MeOH and acetone, slightly soluble in EtOAc and $CHCl_3$, and insoluble in ether, *n*-hexane and H_2O. Compounds **1**, **2** and **3** gave a positive color reaction with molybdophosphoric acid, sulfuric acid, iodine vapor, Ehrlich and Dragendorff reagents, but a negative reaction with ninhydrin reagent. The Rf values of **1**, **2** and **3** on a Silica gel 60 F_{254} precoated glass plate using the solvent system of $CHCl_3$ - MeOH (4 : 1) were 0.73, 0.82 and 0.27, respectively, as a result of exposure to UV light at 254 nm. Compounds **1**, **2** and **3** eluted with a retention time at 15.1 minutes, 9.3 minutes and 17.1 minutes, respectively by HPLC analyses on Supelcosil ABZ+plus with acetonitrile - H_2O (20 : 80) as a mobile phase.

Table 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR chemical shift assignments of quinolactacins A (**1**), B (**2**) and C (**3**) in DMSO-d_6.

	1		**2**		**3**	
Position	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)
1	168.3		168.6		166.3	
2-NH		8.17 (1H, s)		8.09 (1H, s)		8.19 (1H, s)
3	58.9	4.84 (1H, s)	58.5	4.81 (1H, s)	88.4	
3a	164.0		164.3		163.3	
4a	141.2		141.3		141.4	
5	117.0	7.83 (1H, d, J = 8.4 Hz)	117.0	7.82 (1H, d, J = 8.4 Hz)	117.2	7.87 (1H, d, J = 8.4 Hz)
6	132.4	7.81 (1H, dd, J = 8.4, 6.8 Hz)	132.5	7.80 (1H, dd, J = 8.4, 6.8 Hz)	132.7	7.82 (1H, dd, J = 8.4, 6.8 Hz)
7	124.2	7.48 (1H, dd, J = 7.2, 6.8 Hz)	124.3	7.48 (1H, dd, J = 7.2, 6.8 Hz)	124.6	7.50 (1H, dd, J = 7.2, 6.8 Hz)
8	125.8	8.26 (1H, d, J = 7.2 Hz)	125.8	8.25 (1H, d, J = 7.2 Hz)	125.7	8.26 (1H, d, J = 7.2 Hz)
8a	128.0		128.0		128.5	
9	171.5		171.6		171.4	
9a	110.3		110.1		108.8	
4-Me	36.0	3.86 (3H, s)	36.0	3.84 (3H, s)	34.6	4.05 (3H, s)
3-OH						6.93 (1H, s)
1'	35.7	2.19 (1H, m)	29.0	2.45 (1H, m)	40.7	2.25 (1H, m)
2'	20.8	0.83 (1H, m)	13.7 [a]	0.46 (3H, d, J = 6.4 Hz) [a]	23.3	0.83 (1H, m)
		0.88 (1H, m)				0.89 (1H, m)
3'	11.4	0.65 (3H, t, J = 7.4 Hz)			11.3	0.72 (3H, t, J = 7.3 Hz)
1'-Me	17.5	1.14 (3H, d, J = 6.8 Hz)	20.6 [a]	1.14 (3H, d, J = 6.4 Hz) [a]	12.2	1.13 (3H, d, J = 6.8 Hz)

[a] Assignment may be interchanged.

Structure Elucidation

The structural studies were first carried out on the major component, quinolactacin A (**1**), and the structures of quinolactacins B (**2**) and C (**3**) were subsequently determined by comparing the MS and NMR spectral data with those of **1**. The structures of compounds **1**, **2** and **3** were mainly deduced from various NMR spectral analyses including ^{1}H-^{1}H COSY, PFG (pulsed field gradient)-HMQC, PFG-HMBC, NOE difference and NOESY experiments.

Quinolactacin A (1)

The ^{13}C NMR and DEPT spectra of **1** (DMSO-d_6) revealed the presence of six sp^3 carbons, consisting of two methyl carbons (δ_C 11.4 and 17.5), one *N*-methyl carbon (δ_C 36.0), one methylene carbon (δ_C 20.8) and two methine carbons (δ_C 35.7 and 58.9). Additionally, compound **1** contained ten sp^2 carbons, consisting of four olefinic methine carbons (δ_C 117.0, 124.2, 125.8 and 132.4), four olefinic quarternary carbons (δ_C 110.3, 128.0, 141.2 and 164.0) and two carbonyl carbons (δ_C 168.3 and 171.5). The degree of unsaturation of **1** was estimated to be nine by its molecular formula, $C_{16}H_{18}N_2O_2$. The signals of eight olefinic carbons and the two carbonyl carbons account in **1** for six degrees of unsaturation, so the remaining three degrees of unsaturation should be due to the presence of three rings in the molecule. The ^{1}H NMR spectrum of **1** (DMSO-d_6, Table 2) indicated the presence of two methyl protons [δ_H 0.65 (t) and 1.14 (d)], one *N*-methyl proton [δ_H 3.86 (s)], one methylene proton [δ_H 0.83 (m) and 0.88 (m)], two methine protons [δ_H 2.19 (m) and 4.84 (s)], four olefinic protons [δ_H 7.48 (dd), 7.81 (dd), 7.83 (d) and 8.26 (d)] and one deuterium exchangeable NH proton [δ_H 8.17 (s)]. The connectivity of proton and carbon atoms was established by the PFG-HMQC spectrum. Two proton sequences, $-^{5}CH=^{6}CH-^{7}CH=^{8}CH-$ and $-^{3}CH-^{1'}CH(CH_3)-^{2'}CH_2-^{3'}CH_3$, were elucidated from the ^{1}H-^{1}H COSY spectrum. The structure of the remaining part ($C_7H_4N_2O_2$) was determined as follows. The HMBC experiments successfully revealed the presence of a quinolone skeleton with a γ-lactam ring in **1**. In the PFG-HMBC spectra of **1**, the olefinic proton showed correlations from 8-H (δ_H 8.26) to the quaternary olefinic carbon of 4a-C (δ_C 141.2) and a

Fig. 2. ^{1}H-^{1}H COSY and HMBC experiments of quinolactacin A (**1**), B (**2**) and C (**3**).

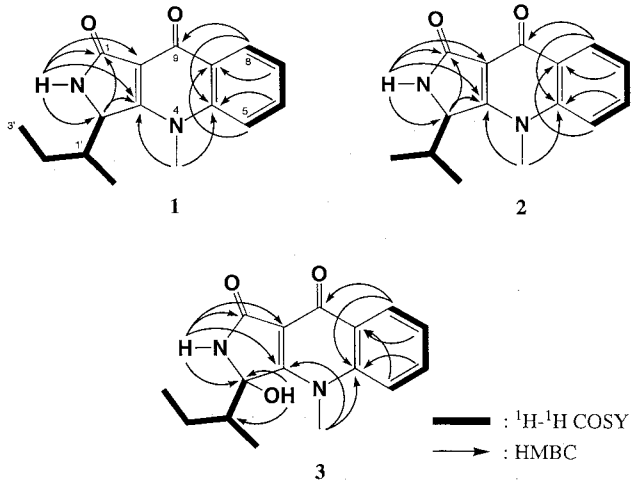
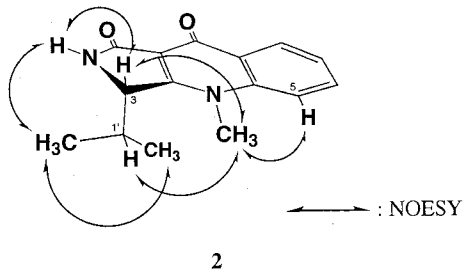


Fig. 3. NOESY experiments of quinolactacin B (**2**).



carbonyl carbon of 9-C (δ_C 171.5), from 7-H (δ_H 7.48) to quaternary olefinic carbon of 8a-C (δ_C 128.0), from 6-H (δ_H 7.81) to 4a-C, and from 5-H (δ_H 7.83) to 8a-C, respectively. Moreover, the *N*-methyl proton signal at δ_H 3.86 (4-CH_3) showed correlations with two quaternary olefinic carbons of 3a-C (δ_C 164.0) and 4a-C. These data suggested the presence of a quinolone skeleton, as shown in Fig. 2. The quinolone skeleton, including a γ-lactam ring, was elucidated from the following correlations. A singlet NH proton signal at δ_H 8.17 (2-NH) was correlated with two quaternary olefinic carbons of 3a-C and 9a-C (δ_C 110.3), a carbonyl carbon of 1-C (δ_C 168.3) and a methine carbon of 3-C (δ_C 58.9). A methine proton signal at δ_H 4.86 (3-H) was correlated with 1-C and 3a-C. The observation of NOE between 4-CH_3 (δ_H 3.86) and 3-H (δ_H 4.84), 4-CH_3 and 5-H (δ_H 7.83), 4-CH_3 and 1′-H (δ_H 2.19), 2-NH (δ_H 8.17) and 3-H, and 2-NH and 1′-CH_3 (δ_H 1.14) supported the above-described predictions. In addition, compound **1** exhibited an ion peak at *m/z* 213 in the EI-MS spectrum, due to loss of the C_4H_9 (*sec*-butyl group) fragment from the molecular ion. The absolute configuration at 3-C of **1** has not yet been established. Based on all of the observations described above, the planar structure of **1** was elucidated to be 3-*sec*-butyl-4-methyl-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinoline-1,9 (4*H*)-dione, as shown in Fig. 1.

Quinolactacin B (**2**)

The molecular formula ($C_{15}H_{16}N_2O_2$) of compound **2** has one less carbon atom and two less protons than that of **1**. The methylene proton and carbon signals corresponding to 2′-CH_2 (δ_C 20.8/δ_H 0.83 and 0.88) of **1** were not observed in the ^{1}H and ^{13}C NMR spectra of **2**. Furthermore, the ^{1}H-^{1}H COSY spectrum revealed the proton sequence of $-^3$CH–$^{1'}$CH(CH_3)–$^{2'}CH_3$. The HMBC spectra of **2** unequivocally showed that compound **2** has a similar basic structure to **1**, but a different side chain, as shown in Fig. 2. The ^{1}H NMR spectrum showed a methyl proton signal with an unusually high magnetic field (δ_H 0.46) due to the anisotropic effect of the chromophore. The structure was also supported by the results of NOESY experiments as follows. The NOE correlations were observed between 4-CH_3 (δ_H 3.84) and 3-H (δ_H 4.81), 4-CH_3 and 5-H (δ_H 7.82), 4-CH_3 and 1′-H (δ_H 2.45), 2-NH (δ_H 8.09) and 3-H, and 2-NH and 2′ or 1′-CH_3 (δ_H 1.14), as shown in Fig. 3. Therefore, the structure of **2** was predicted to be 3-isopropyl-4-methyl-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinoline-1,9 (4*H*)-dione.

Quinolactacin C (**3**)

The molecular formula ($C_{16}H_{18}N_2O_3$) of compound **3** shows the presence of one more oxygen atom than that of **1**. Comparison of the NMR spectral data between **1** and **3** indicated that **3** possesses the same structure as **1**, except for the signal of the oxygenated quarternary carbon of 3-C (δ_C 88.4) observed in **3**. Additionally, a deuterium exchangeable proton signal at δ_H 6.93 was observed in the ^{1}H NMR spectrum. The methyl carbon signal at δ_H 12.2 (1′-Me) was shifted with a high magnetic field relative to that of **1** by an additional γ-gauche effect of hydroxyl oxygen. The structure of **3** was confirmed by ^{1}H-^{1}H COSY and HMBC experiments, as shown in Fig. 2, and was further confirmed by NOESY and FAB-MS experiments (data not shown). Consequently, the structure of **3** was predicted to be 3-*sec*-butyl-3-hydroxy-4-methyl-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinoline-1,9 (4*H*)-dione.

Quinolactacins A (**1**), B (**2**) and C (**3**) were found to be the first pyrrolo[3,4-*b*]quinoline-type compounds isolated from microbial metabolites.

Experimental

General

NMR spectra were measured on JEOL JNM EX-400 and JEOL JNM LA-400 spectrometers with ^{1}H NMR at 400 MHz and ^{13}C NMR at 100 MHz in DMSO-d_6. Chemical shifts are expressed in δ values (ppm) with DMSO-d_6 (δ_C 39.5) as the internal reference for ^{13}C NMR spectra and DMSO-d_6 (δ_H 2.49) as the internal reference for ^{1}H NMR spectra. Standard techniques were used to obtain the ^{1}H-^{1}H COSY, PFG-HMQC, PFG-HMBC and NOESY spectra. The PFG-HMQC and PFG-HMBC experiments were optimized for $^{1}J_{CH}$=145 Hz and $^{2\sim3}J_{CH}$=8.3 Hz, respectively. A mixing time of 750 msec was used in the NOESY experiments. FAB-MS spectra were measured on a JEOL JMS 700T mass spectrometer using 1% glycerol/MeOH as the matrix. Infrared spectra were recorded on Shimadzu FT IR-4200 and Perkin Elmer system 2000 FT-IR spectrophotometers. The samples prepared and mounted as KBr tablets. Ultraviolet spectra were measured on a Shimadzu UV-3100 spectrophotometer in MeOH. Optical rotations were measured on a Horiba SEPA-200 high sensitive polarimeter in DMSO at 25°C. Melting points were recorded on a Yanaco Model MP melting point apparatus and were uncorrected values. Thin layer chromatography was performed using Silica gel 60 F_{254} precoated glass plates [Merck, No. 5715 (0.25 mm)]. Analytical HPLC was carried out using a Waters HPLC equipped with 510 pump, a SPD-6AV variable UV/VIS-detector (Shimadzu), a Waters 741 Data Module and a reverse-phase column (Supelcosil ABZ+plus, Supelco Inc., 4.6 mm i.d.×250 mm).

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