YM-30059, a Novel Quinolone Antibiotic Produced by *Arthrobacter* sp.

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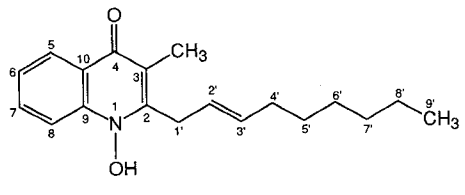
In the course of a screening program, a novel quinolone antibiotic, YM-30059 (Fig. 1) was isolated from the culture broth of *Arthrobacter* sp. YL-02729S. YM-30059 exhibited relatively high antibacterial activities against Gram-positive bacteria including multiple-drug resistant *Staphylococcus aureus* and *S. epidermidis*. The present paper deals with the taxonomy of the producing organism, fermentation, isolation, structure elucidation and biological activity of the new antibiotic.

Microorganism

Strain YL-02729S was freshly isolated from a soil collected from West Kalimantan, Indonesia. Colonies of strain YL-02729S were semi-transparent, white cream, circular and convex on various complex media. By light and electron microscopic observations, strain YL-02729S was Gram-variable rods. A rod-coccus growth change occurred during cultivation in the various complex media. Primary branching occurred after cultivation for 24 to 48 hours, but no true mycelia were produced in any stage of the cell cycle. The temperature range for growth was 15 to 37°C. The cell wall peptide glycan contains lysine as a diamino acid. It was obligately aerobic. Catalase, gelatinase and DNase reactions were positive. The major menaquinones were MK-9 (H2). The mol% of G+C of the DNA was 67.4 (HPLC method [1,2]). Based on the characteristics described above, the strain was identified as a member of the genus *Arthrobacter*, and named *Arthrobacter* sp. YL-02729S.

The type strain has been deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ibaraki Prefecture, Japan, as FERMP P-13102.

Fig. 1. Structure of YM-30059.



Fermentation

A thawed suspension of the producing organism was used to inoculate a 500-ml Erlenmeyer flask containing 100 ml of the seed medium which consisted of glucose 1%, potato starch 2%, yeast extract 0.5%, polypepton (Nihon Pharmaceutical Co., Ltd.) 0.5% and $CaCO_3$ 0.4%, pH 7.3. The flask was incubated at 28°C for 2 days on a rotary shaker at 220 rpm. The seed culture (2 ml) was transferred to 500-ml Erlenmeyer flasks containing 100 ml of the same medium. The fermentation was carried out at 28°C for 3 days on a rotary shaker at 220 rpm.

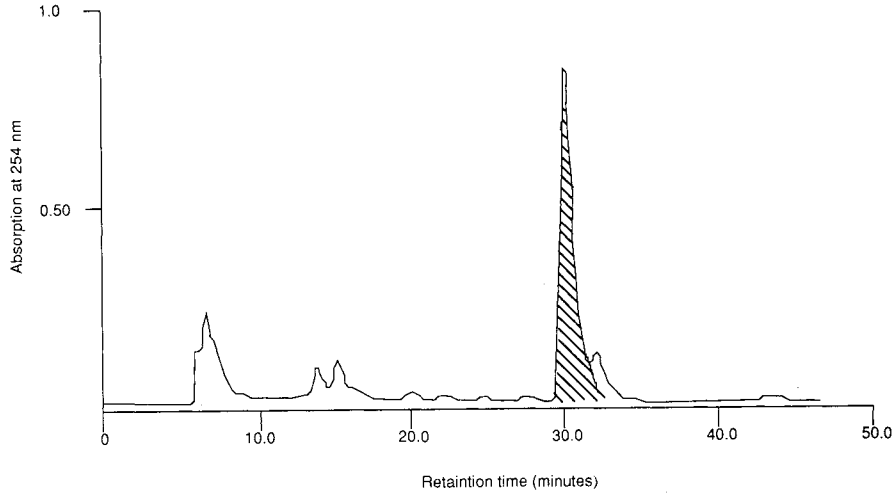
Isolation and Purification

The purification procedure for YM-30059 is outlined in Fig. 2. The fermentation broth of *Arthrobacter* sp. YL-02729S (2.5 liters) was adjusted to pH 7.0 and extracted with ethyl acetate. The organic layer was evaporated *in vacuo* to remove ethyl acetate. The crude extract (2.52 g) was subjected to silica gel column chromatography (24 × 500 mm) eluting with benzene-acetone (7:3) as solvent. The fractions exhibiting antibacterial activity against *S. aureus* FAD 209P were collected and evaporated *in vacuo*. The extract (98.1 mg) was dissolved in methanol and applied to preparative HPLC (column; Shimadzu STR-ODS, 20 × 250 mm, flow rate; 5 ml/minute). The column was developed with methanol - 50 mM phosphate buffer (pH 4.35) - tetrahydrofuran (70:20:10), and the main peak was collected (Fig. 3). The peak fraction was evaporated *in vacuo* to obtain YM-30059 (14.0 mg).

Fig. 2. Isolation and purification procedure for YM-30059.

Fermentation Broth (2.5 liters)
↓ adjusted to pH 7.0, extracted with ethyl acetate
Organic layer
↓ concentrated *in vacuo*
Brown syrup (2.52 g)
↓ Silica gel column chromatography, eluted with benzene - acetone (7:3)
Active fractions (98.1 mg)
↓ ODS-HPLC, eluted with MeOH - 50 mM phosphate buffer (pH 4.35) - tetrahydrofuran (70:20:10), evaporated *in vacuo*
YM-30059 (14.0 mg)

Fig. 3. HPLC profile of YM-30059.



HPLC conditions
Column : Simadzu STR-ODS (20 × 250 mm)
Mobile phase: methanol - 50 mM phosphate buffer (pH 4.35) - tetrahydrofuran (70 : 20 : 10)
Flow rate : 5 ml/minute
Detection : UV 254 nm

* The area of diagonals is the peak of YM-30059.

Table 1. Physico-chemical properties of YM-30059.

Appearance	Yellow syrup
Molecular formula	$C_{19}H_{25}NO_2$
HRFAB-MS (*m/z*)	
Calcd:	300.1964 $(M+H)^+$
Found:	300.1962 $(M+H)^+$
FAB-MS (*m/z*)	300 $(M+H)^+$ 298 $(M-H)^-$
UV λ_{max}^{MeOH} nm (ε)	348 (6,200), 337 (6,300), 250.5 (18,000)
IR ν_{max} (KBr) cm^{-1}	704, 750, 975, 1097, 1251, 1373, 1520, 1589, 1729, 2851, 2917, 3418
TLC Rf value[a]	
$CHCl_3$: MeOH (9 : 1)	0.45
EtOAc : MeOH (9 : 1)	0.47
Benzene : acetone (6 : 4)	0.20
Solubility	
Soluble:	MeOH, DMSO, EtOAc, $CHCl_3$
Insoluble:	H_2O
Color reaction	
Positive:	50% H_2SO_4[b]
Negative:	Ninhydrin

[a] Merck Kieselgel $60F_{254}$.
[b] The color is blue.

Table 2. ^1H and ^{13}C NMR chemical shifts of YM-30059.

Position	δ_C[a]	δ_H[b]
2	152.5	
3	116.8	
4	175.8	
5	126.7	8.28 (d, J=7.5 Hz)
6	125.5	7.40 (t, J=7.5 Hz)
7	133.3	7.69 (t, J=7.5 Hz)
8	116.8	7.97 (d, J=7.5 Hz)
9	141.4	
10	125.2	
1′	33.2	3.76 (2H, s)
2′	124.9	5.59 (m)
3′	135.5	5.58 (m)
4′	34.0	2.01 (2H, m)
5′	30.3	1.25 (2H, m)
6′	30.8	1.34 (2H, m)
7′	33.3	1.28 (2H, m)
8′	24.1	1.25 (2H, m)
9′	14.8	0.85 (3H, t, J=7.0 Hz)
3-Me	12.0	2.21 (3H, s)

[a] 125 MHz, δ in ppm, measured in $CDCl_3$.
[b] 500 MHz, δ in ppm, measured in $CDCl_3$.

Structure Elucidation

The physico-chemical properties of YM-30059 are shown in Table 1. The molecular formula of YM-30059 was determined to be $C_{19}H_{25}NO_2$ (*m/z* found 300.1962 calcd 300.1964) by the high-resolution FAB mass analysis. The ^1H and ^{13}C NMR spectral data are summarized in Table 2. The heteronuclear multiple-bond correlation (HMBC) spectrum displayed ^1H-^{13}C long-range couplings from 5-H to the carbonyl carbon (C-4), from 3-CH_3 to C-2, C-3 and C-4, and from 1′-H to C-3 indicating the connection of $-C^{10}-(C^4=O)-(C^3-CH_3)-C^2-C^{1'}H_2-$. Considering the chemical shifts of C-2 (δ 152.5) and C-9 (δ 141.4), the nitrogen atom was connected between C-2 and C-9. The long-range couplings from 1′-H to C-2′ and C-3′, from 4′-H to C-2′ and C-3′, and from 5′-H to C-3′ indicated the presence of the 2-nonenyl group. The geometry of the disubstituted double bond (C-2′ and C-3′) was determined to be *trans*

Table 3. Antimicrobial spectrum of YM-30059.

Test organisms	MIC (μg/ml)	
	YM-30059	Tosufloxacin
Staphylococcus aureus FDA 209P	6.25	0.1
S. aureus No. 5 (MRSA)*	6.25	>100
S. epidermidis IID 866	6.25	0.1
S. epidermidis No. 17 (MRSE)*	6.25	>100
Bacillus subtilis ATCC 6633	6.25	0.05
Escherichia coli 0-1	>50	0.1
Pseudomonas aeruginosa NCTC 10490	>50	0.2
Klebsiella pneumoniae ATCC 10031	>50	0.013
Mycobacterium smegmatis ATCC 607	12.5	>100

* The clinically isolated strain which shows resistance to β-lactams, macrolides and quinolones.

because the chemical shift of C-4′ (δ 34.0) was over δ 30.0[3]. A daughter ion at *m/z* 283 $(M-OH+H)^+$ in the mass spectrum and the absorption band at 3418 cm^{-1} in the IR spectrum indicated the presence of hydroxyl. From these results, the hydroxyl was attached to N-1. The structure of YM-30059 was determined to be (*E*)-1-hydroxy-3-methyl-2-(2-nonenyl)-4-quinolone.

Biological Activities

The antibacterial activity of YM-30059 is shown in Table 3. MICs were determined by the serial agar dilution method using Mueller-Hinton medium. YM-30059 showed moderate antibacterial activities against Gram-positive bacteria including multiple-drug resistant *S. aureus* and *S. epidermidis*.

The cytotoxic activity of YM-30059 was examined against HeLa S3 cells *in vitro*. HeLa S3 cells were cultured in HAM's F12 supplemented with 10% fetal bovine serum and 20 mM HEPES buffer. When the cells were exposed to the antibiotic for 72 hours in a humidified atmosphere containing 5% CO_2, the value of IC_{50} was 0.59 μg/ml.

Further examinations were conducted to evaluate the biological properties of YM-30059 and revealed that YM-30059 was a potent inhibitor of lipoxygenase[4,5]. KF8940, whose chemical structure is similar to YM-30059, has also been reported as a inhibitor of lipoxygenase[6].

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