Ochracenomicins A, B and C, New Benz[*a*]anthraquinone Antibiotics from *Amicolatopsis* sp.

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In the course of our screening for new antibiotics, new members of benz[*a*]anthraquinone antibiotics, ochracenomicins A (**1**), B (**2**) and C (**3**) (Fig. 1), were isolated from a culture broth of *Amicolatopsis* sp. MJ950-89F4. This strain also produced two known antibiotics, ochromycinone[1] (**4**) and tetrangomycin[2], in the cultured broth. In this report, we wish to report the production isolation, physico-chemical properties, structure determination and biological properties of **1**, **2** and **3**.

A slant culture of the strain MJ950-89F4 on asparagine-glucose agar was inoculated into a 500-ml baffled Erlenmeyer flask containing 110 ml of a seed medium consisting of galactose 2%, dextrin 2%, Bacto-soytone (Difco) 1.0%, corn steep liquor (Iwaki Co.) 0.5%, glycerol 1.0%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% in deionized water (pH 7.4 before sterilization). The culture was incubated on a rotary shaker (180 rpm) at 30°C for 3 days. Two ml of the seed culture was transferred into 500-ml baffled Erlenmeyer flask containing 110ml of a producing medium which was consisting of galactose 2%, dextrin 2%, Bacto-soytone 1.0%, corn steep liquor 0.5%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% in deionized water (pH 7.4 before sterilization). The fermentation was carried out at 27°C for 4 days on a rotary shaker.

The antibiotics were monitored by antibacterial activity against *Staphylococcus aureus* Smith during the purification process.

The fermentation broth (10 liters) was centrifuged at 2,500 rpm for 10 minutes. The mycelial cake was extracted with 2 liters of MeOH. The MeOH extract without evaporation of the solvent, was added to the supernatant. Then it was adjusted to pH 2.0 with 6N HCl. The combined solution applied to a Diaion HP-20 column (500 ml). The active substances were eluted with acetone (2 liters) after washing with water (2 liters) and 50% aq acetone (2 liters). The eluate was concentrated under reduced pressure to give brownish oil (4.3 g). The crude oily material was charged on a column of silica gel (100 ml) and developed with hexane - EtOAc (7 : 3 and 1 : 1, 800 ml). Compound **3** was obtained as pale yellow crystalline (58.4 mg) during concentration of active fractions (Fr. Nos. 13~19, 10 g/Fr.). A mixed fraction of containing compounds **1** and **2** was concentrated to dryness *in vacuo* to yield 145 mg (Fr. Nos. 64~67) of dark yellow solid. The solid was dissolved in hexane-EtOAc and left overnight at room temperature to yield pale yellow crystalline of compound **2** (75.2 mg). Compound **1** was purified by silica gel PTLC (art 5715, Merck) using petroleum ether - EtOAc (7 : 3) as a development solvent (Rf 0.3). Compound **1** was further purified by silica gel PTLC with $CHCl_3$ (Rf 0.38, twice developments). Compound **1** was collected and extracted with $CHCl_3$ - MeOH (1 : 1). The extract was concentrated

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

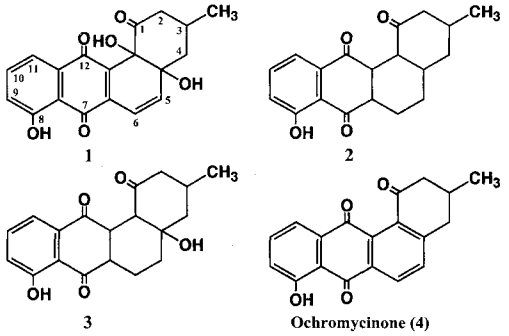

Table 1. Physico-chemical properties of ochracenomicins A (**1**), B (**2**) and C (**3**).

	1	2	3
MP (°C)	195~203 (dec)	187~191 (dec)	199~204 (dec)
Molecular formula	$C_{19}H_{16}O_6$	$C_{19}H_{20}O_4$	$C_{19}H_{20}O_5$
FAB-MS (*m/z*)	340 (M^-)	312 (M^-)	328 (M^-)
$[\alpha]_D^{23}$ $CHCl_3$	+14.0° (*c* 0.2)	−29.5° (*c* 1.0)	−6.8° (*c* 0.5)
UV λ_{max} nm (ε)			
MeOH	214 (31,000), 235 (20,000) sh, 310 (6,900), 412 (5,800)	228 (22,500), 263 (5,700), 345 (5,300)	228 (23,500), 263 (4,700), 344 (5,500)
MeOH - 0.03N NaOH	206 (59,400), 226 (26,600) sh, 283 (13,900), 315 (9,800) sh, 391 (2,900), 540 (4,700)	206 (44,700), 261 (10,000), 399 (3,500)	203 (40,100), 260 (9,500), 396 (4,800)
IR ν_{max} (KBr) cm^{-1}	3600~3300, 2961, 1719, 1660 (sh), 1638, 1620, 1561, 1456, 1362, 1296, 1042	3600~3300, 2970, 1720, 1700, 1650, 1460, 1332, 1272, 1245, 1218, 1165	3600~3300, 2960, 2940, 1710 (sh), 1705, 1644, 1460, 1338, 1280, 1230, 1175, 1095
Color reaction positive	$FeCl_3$, vanilline - H_2SO_4	$FeCl_3$, vanilline - H_2SO_4	$FeCl_3$, vanilline - H_2SO_4

Table 2. ^{1}H and ^{13}C NMR data of ochracenomicins A (**1**), B (**2**) and C (**3**).

No.	1		2		3	
	δ_C[a]	δ_H[b]	δ_C	δ_H	δ_C	δ_H
1	206.8		209.5		207.5	
2	46.5	2.13 (d, 12.0)[c], 2.78 (dt, 2.5, 3.0, 12.0)	50.1	2.36 (t, 10), 2.45 (m)	49.8	2.35 (m), 2.50 (dd, 2.0, 7.0)
3	30.5	2.34 (m)	35.5	1.96 (m)	30.7	2.35 (m)
4	41.2	1.42 (m, 2.5, 11.0, 14.0), 2.09 (dt, 3.0, 3.0, 14.0)	41.7	1.33 (t, 12.0, 12.0), 1.89 (m)	47.6	1.64 (m), 1.85 (m)
4a	78.8	2.98 (d, 2.5, OH)	42.5	1.46 (m)	74.8	
5	146.4	6.43 (d, 9.5)	31.6	1.25 (m), 1.97 (m)	37.3	1.63 (m), 1.88 (m)
6	117.2	6.89 (d, 9.5)	24.8	1.49 (m), 2.45 (m)	19.7	1.99 (m), 2.25 (dq, 3.0, 7.0, 14.0)
6a	138.7		49.1	2.77 (ddd, 4.0, 12.0, 14.0)	48.7	2.76 (ddd, 4.0, 12.0, 13.0)
7	187.9		203.7		203.3	
7a	114.6		117.2		117.1	
8	161.8		161.3		161.3	
9	124.9	7.30 (dd, 1.0, 8.0)	123.3	7.22 (dd, ~1, 7.0)	123.3	7.22 (dd, ~1, 8.0)
10	138.7	7.65 (dd, 7.0, 8.0)	136.8	7.60 (dd, 7.0, 8.0)	136.8	7.60 (dd, 7.0, 8.0)
11	119.6	7.60 (1.0, 7.0)	117.9	7.41 (dd, ~1, 8.0)	117.8	7.38 (dd, 7.0, 8.0)
11a	131.7		136.7		136.9	
12	182.3		196.2		196.7	
12a	138.3		48.8	3.30 (dd, 10.0, 14.0)	45.9	3.56 (dd, 10.0, 13.0)
12b	75.8	4.99 (s, OH)	52.6	2.65 (br t, 10.0, 12.0)	54.4	2.92 (d, 10.0)
3-Me	21.6	1.02 (d, 6.0)	22.4	1.09 (d, 7.0)	22.0	1.12 (d, 6.0)

[a] 500 MHz ($CDCl_3$, ref TMS).
[b] 125 MHz ($CDCl_3$, ref TMS).
[c] Multiplicity, coupling constant (Hz).

in vacuo yielding orange prism of **1** (7.8 mg).

The physico-chemical properties of **1**, **2** and **3** are summarized in Table 1. The molecular formulas of **1**, **2** and **3** were established as $C_{19}H_{16}O_6$, $C_{19}H_{20}O_4$ and $C_{19}H_{20}O_5$ respectively, on the basis of FAB-MS , ^{1}H and ^{13}C NMR spectra. The ^{1}H and ^{13}C NMR spectral data of **1**, **2** and **3** are shown in Table 2.

The IR spectrum of compound **1** indicated the presence of ketone carbonyl (1719 cm^{-1}), chelated quinone carbonyl (1638 cm^{-1}) and non-chelated quinone carbonyl (1660 cm^{-1}, sh). The UV spectra and color reaction with ethanolic $FeCl_3$ of **1** suggested the presence of perihydroxy-naphthoquinone chromophore in its structure. The ^{1}H-^{1}H COSY spectrum of **1** revealed the presence of a trisubstituted benzene and two partial structures as follows: $-CH=CH-$ and $-CH_2-CH(CH_3)-CH_2-$. The color reaction and partial structures closely resemble to those of ochromycinone (**4**), which belongs to benz[*a*]anthraquinone antibiotic group.

The connectivity among the partial structures of **1** was performed by HMBC experiment. In the HMBC spectrum of **1**, two hydroxyl protons at δ_H 2.98 (4a-OH) and δ_H 4.99 (12b-OH) were coupled to two sp^3 quaternary carbons at δ_C 78.7 (C-4a) and δ_C 75.8 (C-12b), respectively. The 4a-OH proton was also coupled to a methylene carbon at δ_C 41.2 (C-4). Another methylene protons at δ_H 2.13 (2-Ha) and δ_H 2.78 (2-Hb) were coupled to a carbonyl carbon at δ_C 206.8 (C-1) and the sp^3 quaternary carbon (C-12b). An olefinic proton at δ_H 6.39 (6-H) was coupled to a quinone carbonyl carbon at δ_C 187.9 (C-7) and the quaternary carbons at δ_C 78.7 (C-4a) and δ_C 138.3 (C-12a). The result of HMBC experiment of **1** is summarized in Fig. 2. From the above mentioned results, the structure of **1** was determined to

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

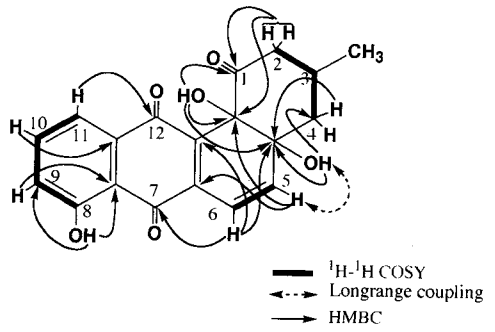

Fig. 3. Summary of ^{1}H-^{1}H COSY and HMBC experiments of **2** and **3**.

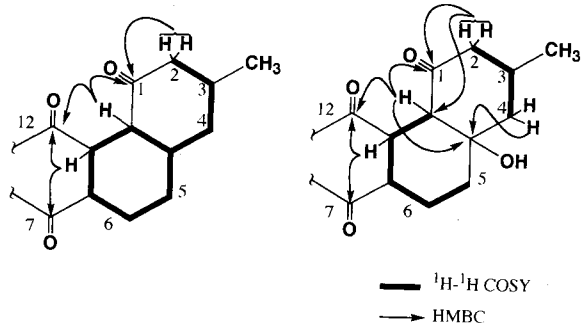

Table 3. Antibacterial activities of ochracenomicins A (1), B (2) and C (3).

Test organism	MIC (μg/ml)		
	1	2	3
Staphylococcus aureus FDA209P	1.56	>100	50
S. aureus Smith	0.78	>100	50
S. aureus MS 9610	1.56	>100	50
S. aureus MS 16526 (MRSA)	1.56	>100	50
S. aureus TY-04282 (MRSA)	1.56	>100	50
Micrococcus luteus IFO 3333	0.39	>100	100
M. luteus PCI 1001	0.39	>100	50
Bacillus subtilis NRRL B-558	1.56	>100	50
B. cereus ATCC 10702	3.12	>100	50
Corynebacterium bovis 1810	6.25	>100	100
Escherichia coli NIHJ	6.25	>100	>100
Shigella dysenteriae JS 11910	3.12	>100	100
Salmonella enteritidis	25	>100	>100
Proteus mirabilis IFM OM-9	6.25	>100	>100
Providencia rettgeri GN 466	25	>100	>100
Serratia marcescens	100	>100	>100
Pseudomonas aeruginosa A3	100	>100	>100
Klebsiella pneumoniae PCI 602	25	>100	100
Mycobacterium smegmatis ATCC 607	50	>100	50
Candida albicans 3147	25	>100	100

be 3,4,4a,12b-tetrahydro-4a,8,12b-trihydroxy-3-methylbenz[*a*]anthracene-1,7,12(2*H*)-trione.

The ^{1}H and ^{13}C NMR spectra of **2** and **3** indicated that these structures to be very similar to compound **1** except for the carbon signals at α position of quinone carbonyl (C-6a and C-12a) and olefinic carbons (C-5 and C-6) in **1**. Judging from the analyses of the 2D NMR experiments in **2** and **3**, it was concluded that the α position carbons of quinone carbonyl and olefinic carbons were reduced to the methine and methylene carbons, respectively, in compounds **2** and **3** (Fig. 3). Thus, the structures of compound **2** and **3** were determined as shown in Fig. 1. These antibiotics are new family of benz[*a*]anthraquinone antibiotics ochromycinone, fujimanmycin[3] and SF2315[4].

The antimicrobial activities of compound **1**, **2** and **3** are shown in Table 3. Compound **1** showed broad and strong antimicrobial activities against Gram-positive bacteria including MRSA, some Gram-negative bacteria and *Candida albicans* 3147. The MICs of compound **2** has no activities against Gram-positive, Gram-negative bacteria and yeast like fungi. Compound **3** has weak activity against Gram-positive bacteria and no activities against Gram-negative bacteria and yeast like fungi. The acute toxicity (LD_{100}, ip) of **1** in mice was <6.25 mg/kg.

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