Chrolactomycin, a Novel Antitumor Antibiotic Produced by *Streptomyces* sp.

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In the course of our screening for antitumor antibiotics, a novel compound, chrolactomycin, was isolated from *Streptomyces* sp. 569N-3. Chrolactomycin exhibited antimicrobial activity and antiproliferative activity against human tumor cell lines. In this paper, we describe the fermentation, isolation, physico-chemical properties, structure elucidation and biological activities of chrolactomycin.

A loopful of the cells from a mature slant of strain 569N-3 was inoculated into each of two 300-ml Erlenmeyer flasks containing 30 ml of the seed medium composed of glucose 1%, soluble starch 1%, Bacto tryptone 0.5%, beef extract 0.3%, yeast extract 0.5% and $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.05% in deionized water (pH 7.2 prior to sterilization). The inoculated flask content was incubated on a rotary shaker at 28°C for 3 days. 7.5 ml of the seed culture was added to a 1-liter Erlenmeyer flask containing 150 ml of the same medium. Following 2 days of incubation at 28°C, one hundred fifty ml of the second stage seed culture was transferred into a 5-liter jar fermenter containing 3 liters of the same medium. After incubation on a fermenter at 28°C for 1 day, nine hundred ml of the third stage seed culture was transferred into a 30-liter jar fermenter containing 18 liters of a fermentation medium composed of sucrose 5%, dry yeast 1.5%, KH_2PO_4 0.05%, $Mg_2SO_4 \cdot 7H_2O$ 0.05% and $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.05% in deionized water (pH 7.0 prior to sterilization). The fermentation was carried out at 28°C for 8 days with agitation of 150 rpm and aeration of 18 liters per minute.

The culture broth (13.8 liters) was divided into culture filtrate and mycelial cake by a centrifugal filter. The mycelial cake was extracted with MeOH. After filtration,

Table 1. Physico-chemical properties of chrolactomycin.

Appearance	White powder
Melting point	130-131° C
$[\alpha]_D^{28.5}$ (c 0.45, MeOH)	-7.75°
Molecular formula	$C_{24}H_{32}O_7$
FAB-MS (m/z)	433 $[M+H]^+$, 431 $[M-H]^-$
HRFAB-MS (m/z)	
Found	431.2087 $[M-H]^-$
Calcd.	431.2069 (for $C_{24}H_{31}O_7$)
UV λ_{max} nm (ε) (MeOH)	209.8 (4738)
IR ν_{max} (KBr) cm^{-1}	3800-2400, 3410, 2954, 2929, 2866, 1793, 1712, 1689, 1641, 1626, 1458, 1255, 1186, 1178, 1113, 1074, 966
TLC (Rf value[a])	0.41
Solubility	
Soluble	MeOH, Me_2CO, AcOEt, $CHCl_3$, DMSO
Insoluble	Hexane

[a] Silica gel TLC (Kieselgel 60 F_{254}, Merck), solvent: $CHCl_3$ - MeOH (19 : 1).

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the MeOH extract was combined with the culture filtrate and the mixture was applied to a column of Diaion HP-20 (2 liters, Mitsubishi Chemical Industries). The column was washed with deionized water - MeOH (2 : 8) and eluted with MeOH (6 liters). The eluate was concentrated and extracted with $CHCl_3$ at pH 3.0. The extract was concentrated *in vacuo* to yield a brown oil. The oil was applied to a column of silica gel Wakogel C-200 (Wako Pure Chemical Industries). The column was washed with $CHCl_3$ - MeOH (800 : 1) and the active substance was eluted with $CHCl_3$ - MeOH (200 : 1). The eluate was concentrated *in vacuo* and then applied to a column of Diaion HP-20SS. The column was eluted with deionized water - acetonitrile (1 : 1) followed by a gradient of deionized water - acetonitrile (45 : 55~30 : 70). This eluate was concentrated *in vacuo* and then applied to a column of silica gel LiChroprep Si 60 (Merck). The column was washed with hexane - acetone (86 : 14) followed by hexane - acetone (85 : 15~78 : 22) to elute the active fractions which were combined and concentrated to yield 325 mg of chrolactomycin as a white powder.

The physico-chemical properties of chrolactomycin are summarized in Table 1. The molecular formula of chrolactomyin was found to be $C_{24}H_{32}O_7$ by HRFAB mass measurement. The ^1H and ^{13}C NMR data of chrolactomycin are shown in Table 2. The ^{13}C NMR data and a DEPT experiment revealed the presence of four methyl carbons, six methylene carbons, seven methine carbons and seven quaternary carbons. The molecular formula agreed well with these data requiring one H_2O-exchangeable proton. ^1H-^1H COSY, HSQC (Hetronuclear Single Quantum Coherence spectroscopy) and HMBC experiments revealed the partial structure of chrolactomycin as shown in Fig. 1. There was no cross peak from the carbonyl carbon (d_c 168.88) in the HMBC experiment. However, of the remaining atoms, CO_2, the chemical shifts of C-13 (d_c

Table 2. ^1H and ^{13}C NMR data of chrolactomycin in $CDCl_3$.

Position	^1H chemical shift	^{13}C chemical shift
1	2.34 (1H, m)	42.67 (d)
2	1.25 (1H, m)	32.11 (t)
	1.61 (1H, m)	
3	1.62 (2H, m)	23.77 (t)
4	1.03 (1H, m)	37.58 (t)
	1.21 (1H, m)	
5	1.79 (1H, m)	30.35 (d)
6	0.94 (1H, m)	45.06 (t)
	1.55 (1H, dd, J=9.6, 14.3Hz)	
7	1.95 (1H, m)	34.50 (d)
8	4.26 (1H, br. d, J=9.8Hz)	82.09 (d)
9		143.71 (s)
10		190.05 (s)
11		78.55 (s)
12	4.53 (1H, s)	83.96 (d)
13		83.36 (s)
14	1.34 (1H, ddd, J=2.0, 6.7, 14.9Hz)	34.34 (t)
	1.76 (1H, dd, J=10.5, 14.9Hz)	
15	2.71 (1H, m)	27.19 (d)
16		132.90 (s)
17	6.83 (1H, dd, J=1.8, 6.0Hz)	140.88 (d)
18		171.04 (s)
19	1.12 (3H, d, J=6.8Hz)	20.14 (q)
20		168.88 (s)
21	3.60 (3H, s)	54.63 (q)
22	5.67 (1H, d, J=1.7Hz)	121.08 (t)
	6.41 (1H, d, J=1.0Hz)	
23	1.08 (3H, d, J=6.6Hz)	20.47 (q)
24	0.89 (3H, d, J=6.8Hz)	23.60 (q)

83.36), indicated the connectivity of the oxygen atom, and an absorption band of 1793 cm^{-1} for γ-lactone in the IR spectrum, thus the structure of chrolactomycin was determined as shown in Fig. 2.

In a NOESY experiment, NOEs between the methoxyl protons, 8-H and 12-H, between 23-H methyl protons and 8-H, and between 12-H and 1-H were observed. These connections show that the methoxyl group, 8-H, 12-H, 23-H methyl group and 1-H were on the same plane. An analysis of other NOEs and coupling constants have indicated the structure of chrolactomycin as shown in Fig. 3 and the configuration is the same as that of okilactomycin[1,2].

Fig. 1. Partial structure of chrolactomycin.

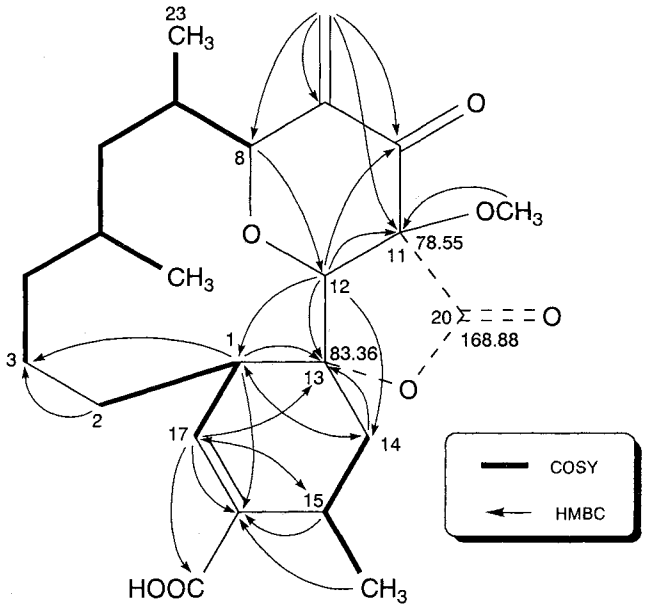


Fig. 3. Relative stereochemistry of chrolactomycin.

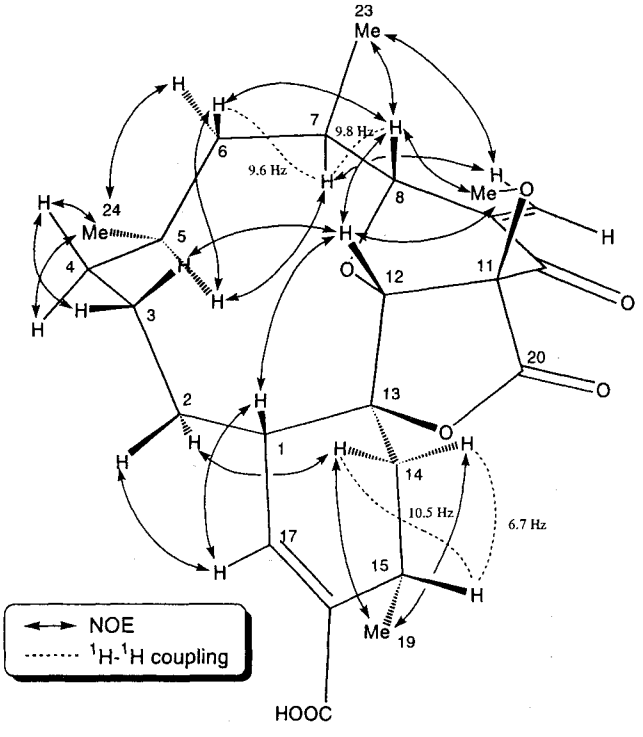
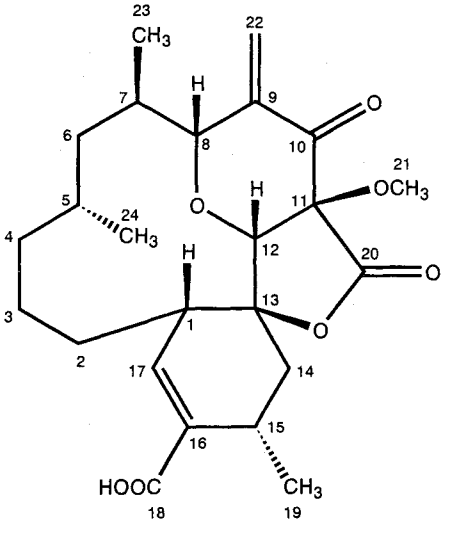
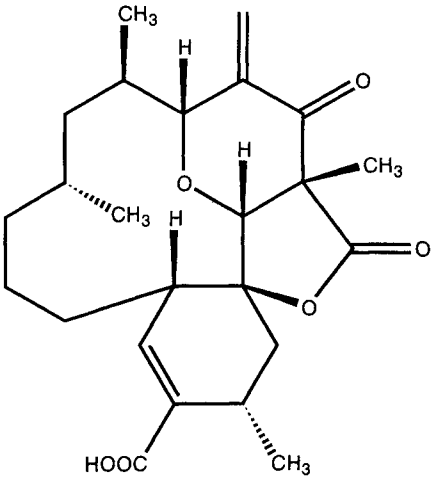


Fig. 2. Structure of chrolactomycin and okilactomycin.



Chrolactomycin



Okilactomycin

Table 3. Antimicrobial activities of chrolactomycin.

Test microorganisms	MIC (μg/ml)
Staphylococcus aureus subsp. *aureus* ATCC 6538 P	5.2
Bacillus subtilis No.10707	5.2
Enterococcus hirae ATCC 10541	10.4
Proteus vulgaris ATCC 6897	>83
Klebsiella pneumoniae subsp. *pneumoniae* ATCC 10031	>83
Escherichia coli ATCC 26	>83
Pseudomonas aeruginosa BMH No.1	>83
Shigella sonnei ATCC 9290	>83
Candida albicans ATCC 10231	>83

Table 4. Antiproliferative activities of chrolactomycin.

Cell lines	IC_{50} (μM)	
	Chrolactomycin	VP-16
ACHN	1.2	0.33
A431	1.6	0.48
MCF-7	0.69	0.57
T24	0.45	1.2

The antimicrobial activity of chrolactomycin is shown in Table 3. Chrolactomycin exhibited antimicrobial activity against Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus hirae* with the MIC values ranged from 5.2 μg/ml to 10.4 μg/ml, but was inactive against Gram-negative bacteria and *Candida albicans*. According to the report of okilactomycin, comparison of the antimicrobial activities against *Staphylococcus aureus* supposes that chrolactomycin may be five times as potent as okilactomycin

The antiproliferative activity of chrolactomycin is shown in Table 4. Chrolactomycin exhibited antiproliferative activity against human tumor cell lines with IC_{50} values ranging from 0.45 μM to 1.6 μM after 72 hours exposure. The antiproliferative activity of chrolactomycin was comparable to that of VP-16 (etoposide). The fact that okilactomycin exhibited antitumor activity against Ehrlich ascites carcinoma[1] suggests that chrolactomycin may have antitumor activity *in vivo*. Detailed studies on the mechanism of action and antitumor activity of chrolactomycin are in progress.

Acknowledgments

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