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Gilvusmycin, a New Antitumor Antibiotic Related to CC-1065

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A new antitumor antibiotic gilvusmycin was isolated from the culture broth of *Streptomyces* sp. QM16. The structure of gilvusmycin was related to CC-1065 and determined by NMR spectral analysis. Gilvusmycin exhibited antitumor activity against murine leukemia P388 *in vivo*.

In the course of screening for new antitumor antibiotics, *Streptomyces* sp. QM16 was found to produce a novel antitumor antibiotic gilvusmycin (**1**). **1** was recovered from both mycelium and filtered broth by extraction and purified by chromatography. Structural studies revealed it to possess similarity to CC-1065[1] (**2**) (Fig. 1).

1 showed growth inhibition against various tumor cells *in vitro*, and also showed antitumor activity against murine leukemia P388 *in vivo*.

In this paper, we describe the production, isolation, structural elucidation and biological properties of **1**.

Taxonomy of the Producing Strain

Culture QM16 was isolated from a soil sample collected at Takahashi, Okayama Prefecture, Japan. Characterization of the strain was carried out mainly by the methods described by SHIRLING and GOTTLIEB[2]. The aerial mycelium of the strain monopodially branched on the main stem and terminated in spirals forming spore chains with more than 50 spores per chain. The spores were cylindrical ($0.5\sim0.6\times0.7\sim1.0\,\mu$m) with smooth surfaces. The cultural and physiological properties of strain QM16 grown on various media at 27°C are shown in Tables 1 and 2, respectively. The whole-cell hydrolysate contained the L, L isomer of diaminopimelic acid which corresponds to cell-wall type 1. Based on these morphological and chemotaxonomic characteristics, it was concluded that the strain belongs to the genus *Streptomyces*.

Fermentation

A well grown agar slant of *Streptomyces* sp. QM16 was used to inoculate a 500-ml Erlenmeyer flask con-

Fig. 1. Structure of gilvusmycin (**1**) and CC-1065 (**2**).

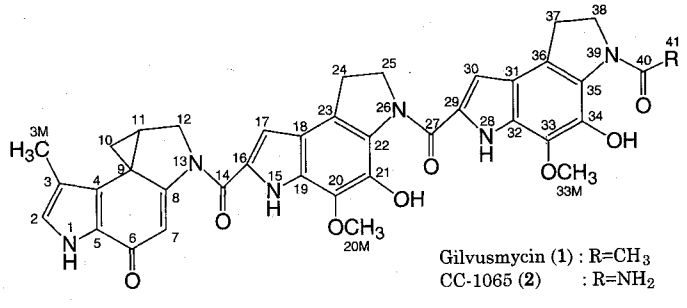


Table 1. Cultural characteristics of strain QM16.

Sucrose-nitrate agar	G:	Good
	Am:	Good; Yellowish gray
	R:	Pale yellowish brown
	Sp:	None
Glycerol-asparagine agar	G:	Good
	Am:	Good; Yellowish gray
	R:	Deep red
	Sp:	None
Yeast extract - malt extract agar	G:	Good
	Am:	Good; Yellowish gray
	R:	Dark yellow
	Sp:	None
Oatmeal agar	G:	Good
	Am:	Good; Yellowish gray
	R:	Yellowish orange
	Sp:	None

G: growth, Am: aerial mycelium, R: reverse side of colony, Sp: soluble pigment.

Table 2. Physiological properties of strain QM16.

Temperature for growth	15~35℃
Production of melanoid pigments	
Tyrosine agar	Negative
Peptone-yeast extract-iron agar	Negative
Tryptone-yeast extract agar	Negative
Hydrolysis of starch	Negative
Liquefaction of gelatin	Negative
Coagulation of milk	Negative
Peptonization of milk	Negative
Reduction of nitrate	Negative
Utilization of carbon source:	
Utilized	L-arabinose, D-xylose, D-glucose, D-fructose, inositol, L-rhamnose, raffinose, D-mannitol
Moderately utilized	sucrose

taining 100 ml of fermentation medium consisting of potato starch 1%, glucose 1%, glycerol 1%, polypeptone 0.5%, yeast extract 0.2%, corn steep liquor 1%, NaCl 0.1% and $CaCO_3$ 0.32%, the pH being adjusted to 7.4 before sterilization. The fermentation was carried out at 27°C for 5 days with shaking on a rotary shaker at 200 rpm.

Isolation and Purification

The isolation was guided by a bioassay (*in vitro* cytotoxicity against P383 cells).

The fermentation broth (1 liter) was centrifuged to give supernatant and mycelium cake. The mycelium cake was extracted with acetone (0.5 liter). The extract was filtered and concentrated *in vacuo* to an aqueous solution. The solution was mixed with the supernatant and

Fig. 2. Isolation scheme of gilvusmycin.

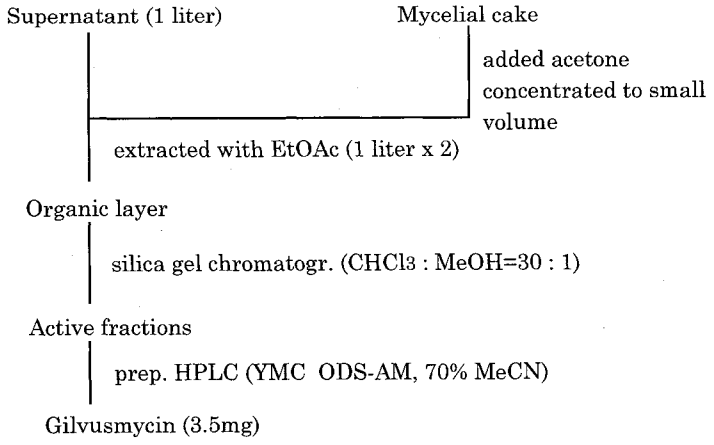


Table 3. Physicochemical properties of gilvusmycin.

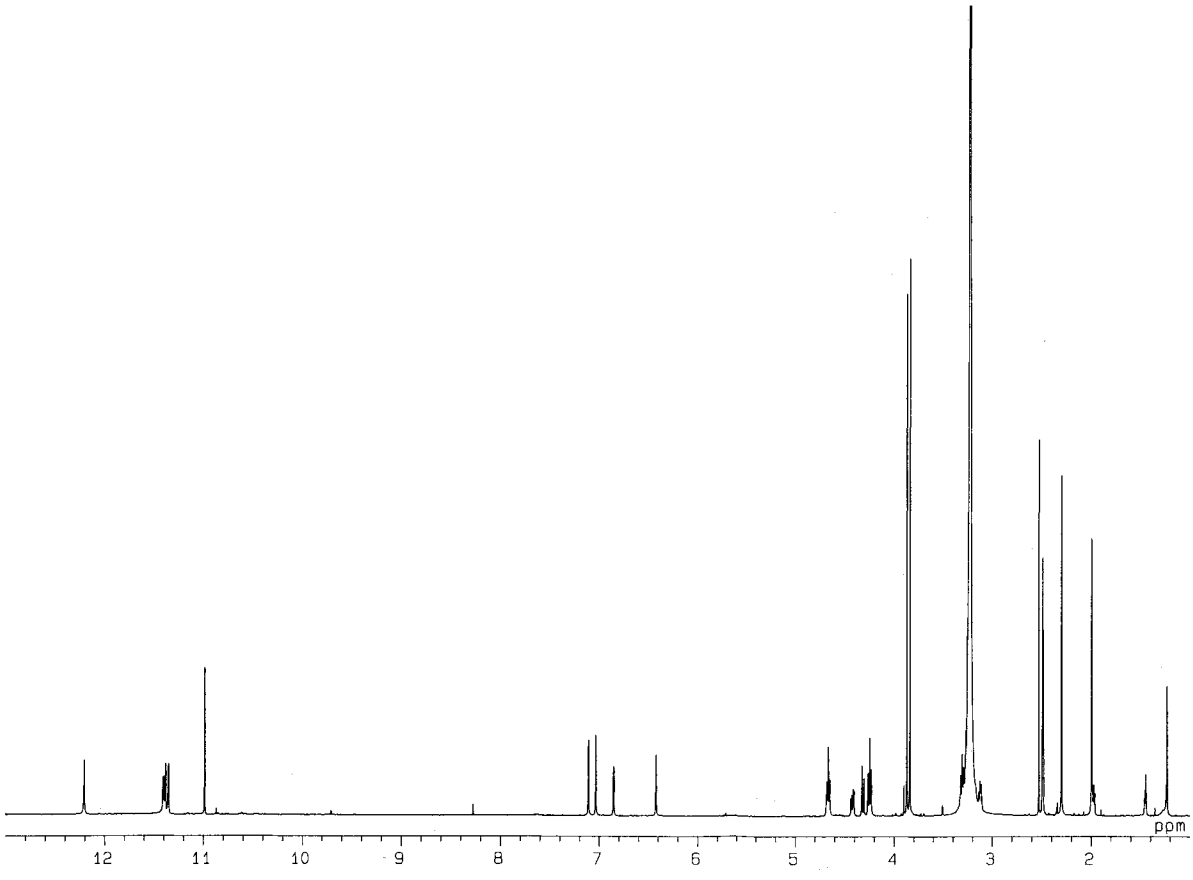
Appearance	Yellow powder
MP	160~162 ℃ (dec.)
$[a]_D^{20}$	+85° (*c* 0.1, DMF)
Molecular formula	$C_{38}H_{34}O_8N_6$
HRFAB-MS Calcd:	703.2516
Found:	703.2515 $(M+H)^+$
UV l_{max} (e) (in MeOH)	276 (27,500), 370 (33,300)
IR n (KBr) cm^{-1}	3446, 1660, 1572, 1522, 1385, 1306, 1269, 1132, 588

extracted twice with 1 liter of ethyl acetate without adjusting pH. After evaporation, the residue was applied to a silica gel column (Wakogel, C-200, 3 × 30 cm) which was developed with chloroform - methanol (30 : 1). The active eluate was concentrated to dryness, solubilized in DMSO and then subjected to preparative HPLC (YMC ODS-AM, i.d. 2 cm × 25 cm, 70% aq MeCN). The active fractions were concentrated to dryness to give a yellow powder of **1** (3.5 mg). The isolation scheme is shown in Fig. 2.

Structural Elucidation

The physico-chemical properties of **1** are summarized in Table 3. The molecular formula of **1** was established as $C_{38}H_{34}O_8N_6$ on the basis of HRFAB-MS data. In the IR spectrum, **1** showed broad strong bands from 1630 to 1700 cm^{-1} suggesting the prerence of several carbonyl functions in **1**. The ^1H NMR spectrum taken in DMSO-d_6 (Fig. 3) revealed the presence of cyclopropane-derived methylene resonance (δ_H 1.42, 1.98), two methyl resonances (δ_H 2.00, 2.30), six methylene resonances (δ_H 3.25, 3.31, 4.25, 4.32, 4.45, 4.64), two methoxy resonances (δ_H 3.84, 3.88), four olefinic methine resonances (δ_H 6.42, 6.86, 7.04, 7.11), two hydrogen-bound phenolic hydroxyl resonances (δ_H 11.00, 12.20) and three imine resonances (δ_H 11.36, 11.38, 11.41). The ^{13}C NMR spectrum demonstrated 38 signals which were assigned to four methyls, six methylenes, five methines and 23 quaternary carbons by DEPT experiment. The

Fig. 3. 50 MHz ^{1}H NMR spectrum of gilvusmycin in DMSO-d_6.



^{1}H and ^{13}C NMR spectral data for **1** are summarized in Table 4.

The characteristic UV spectrum of **1** suggested that **1** had considerable similarity to **2**[3]. Comparison of NMR spectral data of **1** and **2**[4] also supported it. Analyzing the difference, in ^{1}H NMR, **1** had an additional resonance of singlet methyl at δ_H 2.30, and in ^{13}C NMR, the resonance of carbamoyl group at δ_C 157.5 in **2** was absent in **1**, in exchange of that, **1** had two resonances, one is of carbonyl carbon at δ_C 169.3 and the other is of singlet methyl at δ_C 24.0. The analysis above and the difference of molecular formula ($C_{38}H_{34}O_8N_6$: **1**, $C_{37}H_{33}O_8N_7$: **2**) strongly suggested that carbamoyl group in **2** was exchanged to acetyl group in **1**. The HMBC[5] experiments on **1** proved that the linkages of C-1 ~ C-39 in **2** were conserved in **1**. Additionally, a long range coupling from 41-CH_3 to C-40 (carbonyl carbon) was also observed to support the existence of acetyl group in **1**. Furthermore, NOE between 41-CH_3 and 38-CH_2 proved that the acetyl group was attached to the nitrogen at 39. For all these findings, the total structure of **1** was deduced as shown in Fig. 1.

Biological Activity

1 was tested for its *in vitro* cytotoxicity. IC_{50} (μg/ml) values against P388 (murine leukemia), K562 (human chronic myelogenous leukemia), A431 (human epidermoid carcinoma) and MKN28 (human gastric carcinoma) are shown in Table 5. Because of the outstanding cytotoxicity to P388, antitumor activity of **1** was assessed in P388-bearing mice and the results are shown in Table 6. **1** exhibited significant antitumor activity.

Experimental

General

Specific rotation was obtained on a Jasco DIP-140

Table 4. 125 MHz ^{13}C NMR and 500 MHz ^{1}H NMR spectral data of gilvusmycin (**1**) and CC-1065[a] (**2**) taken in DMSO-d_6.

Position	1		2
	δ_C	δ_H	δ_C
1-NH		11.41 (d, 2,5[b])	
2	123.3 d	6.86 (d, 2.5)	123.5
3	112.8 s		113.0
3M	9.4 q	2.00 (s)	9.5
4	126.7 s		129.5
5	128.8 s		157.5
6	176.2 s		176.4
7	110.4 d	6.42 (s)	110.6
8	160.5 s		160.7
9	31.4 s		31.5
10	21.0 t	1.42 (dd, 4.4, 4.4)	21.6
		1.98 (dd, 4.4, 4.4)	
11	20.7 d	3.12 (ddd, 4.4, 4.4, 5.0)	20.9
12	54.5 t	4.32 (d, 11.0)	49.4
		4.45 (dd, 5.0, 11.0)	
14	161.2 s		161.2
15-NH		11.38 (d, 1.8)	
16	129.6[c] s		127.2
17	105.7 d	7.04 (d, 1.8)	105.9
18	117.6 s		117.3
19	130.3[c] s		130.4
20	133.0 s		
20M	60.1 q	3.88 (s)	60.0
21	138.2 s	11.00 (OH)	132.4
22	127.2 s		128.9
23	120.6 s		121.3
24	27.4 t	3.31 (dd, 8.0, 9.5)	26.6
25	53.1 t	4.64 (t, 8.0)	53.3
27	160.0 s		160.2
28-NH		11.36 (d, 2.5)	
29	130.3[c] s		127.5
30	106.4 d	7.11 (d, 2.5)	106.3
31	117.2 s		117.7
32	130.6[c] s		130.7
33	132.4 s		
33M	59.9 q	3.84 (s)	60.3
34	138.2 s	12.20 (OH)	133.1
35	127.0 s		129.1
36	121.1 s		118.2
37	26.4 t	3.25[d]	27.6
38	50.8 t	4.25 (t, 8.0)	54.8
40	169.3 s		157.5
41	24.0 q	2.30 (s)	

[a] Cited from the data by Martin *et al*[4].
[b] Coupling constants in J=Hz.
[c] The assignments may be interchanged.
[d] Resonance in one-dimentional spectra obscured by overlapping signals.

spectropolarimeter. Mass spectra were measured on a JEOL JMS-SX102A in the FAB mode using glycerol matrix. UV and IR spectra were recorded on a Hitachi U-3200 spectrophotometer and a Jasco A-3 spectrophotometer, respectively. NMR spectra were obtained on a JEOL JNM-α500 spectrophotometer with ^{1}H NMR at 500 MHz and ^{13}C NMR at 125 MHz. Chemical shifts are given in ppm using TMS as internal standard.

Table 5. *In vitro* cytotoxicity of gilvusmycin.

Cell line	IC_{50} (ng/ml)
P388	0.08
K562	0.86
A431	0.72
MKN28	0.75

Table 6. *In vivo* antitumor activity of gilvusmycin against P388 murine leukemia.

Dose (mg/kg/day)	MST (days)	T/C (%)
0 (Control)	11.0±0.9	100
0.11	9.8±3.3	89.1
0.037	14.4±3.1	130.9
0.0123	12.2±0.5	110.9

MST: Mean Survival Time.
P388 was implanted ip into CDF_1 mice (1.0×10^6 cells/mouse) on the day 0, then /gilvusmycin was given ip on the days 1, 5 and 9.

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