Phencomycin, a New Antibiotic from a *Streptomyces* Species HIL Y-9031725

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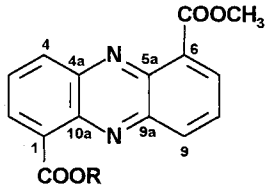
In the course of our screening directed towards the discovery of new antibacterial antibiotics, we isolated a new antibiotic phencomycin (**1**) from the culture filtrate of a fermented *Streptomyces* sp. Y-9031725[1]. In this paper, we report the production, isolation, structure elucidation and biological properties of phencomycin (**1**).

Strain HIL Y-9031725 was isolated from a soil sample collected in India. The strain was identified as belonging to the genus *Streptomyces* using the methods described by SHIRLING and GOTTLIEB.[2] A loopful of mature slant culture of *Streptomyces* Y-9031725 was inoculated into Erlenmeyer flasks (500 ml capacity) containing 100 ml of seed medium consisting of glucose 1.5%, soyabean meal 1.5%, corn steep liquor 0.5%, $CaCO_3$ 0.2% and NaCl 0.5%, pH 6.5 before autoclaving. The flasks were cultivated at 27°C on a rotary shaker at 240 rpm for 72 hours. The resultant seed culture (9%) was inoculated into 15 liter fermenter containing 10 liters of production medium consisting of glucose 1.5%, soybean meal 1.5%, corn steep liquor 0.5%, $CaCO_3$ 0.2% and NaCl 0.5%, pH 6.5 before autoclaving. Desmophen (4 ml) was added as an antifoaming agent to the contents of the fermenter. The fermentation was carried out at 27°C under stirred conditions at 150 rpm with aeration at a rate of 10 liters/minute for 45 hours. The production of the antibiotic was monitored by its antibacterial activity against *Staphylococcus aureus* 209P.

The culture filtrate (8 liters), obtained after separating mycelium by centrifugation, was passed through a column of HP-20 (400 ml). The column was washed with demineralized water (5 liters) and eluted with 50 : 50 water - methanol (5 liters) and 20 : 80 water - methanol (8 liters) respectively. The active eluates were concentrated under reduced pressure to 200 ml. It was then acidified to pH 5 and extracted with ethyl acetate (3 × 150 ml). The combined ethyl acetate extracts were concentrated to dryness under reduced pressure. The crude material (850 mg), thus obtained, was subjected to a silica gel (200 ~ 300 mesh, 150 g) column and eluted with dichloromethane - ethyl acetate mixture. The active eluates, which eluted out in 0.5 ~ 1% ethyl acetate in dichloromethane, were concentrated to dryness followed by crystallization in dichloromethane to obtain pure phencomycin (**1**) (150 mg).



1 R = H
2 R = CH_3

Table 1. Physico-chemical characteristics of phencomycin (**1**).

	1
Appearance	Yellow crystalline solid
Solubility	CH_2Cl_2, $CHCl_3$, CH_3CN, EtOAc, MeOH and DMSO
Molecular weight (EI-MS)	282
Molecular formula	$C_{15}H_{10}N_2O_4$
Elemental analysis	
Found:	C 63.77, H 3.60, N 9.89
Calcd:	C 63.82, H 3.54, N 9.93
TLC (SiO_2) Rf	0.43[a]
HPLC RT	11.63 minutes[b]
UV (MeOH) nm	254, 368
(MeOH + HCl) nm	250, 371
(MeOH + NaOH) nm	258, 366
IR (KBr) cm^{-1}	3450, 1720, 1700, 1615, 1530, 1415, 1400, 1350, 1320, 1280, 1260, 1200, 1185, 1030, 915, 860, 830, 800 and 735
^1H NMR (300 MHz, $CDCl_3$, δ)	15.11 (br s, 1H), 9.02 (dd, 7.02, 1.22 Hz, 1H), 8.63 (dd, 8.16, 1.22 Hz, 1H), 8.44 (dd, 8.16, 1.22 Hz, 1H), 8.39 (dd, 7.02, 1.22 Hz, 1H), 8.08 (dd, 8.16, 7.02 Hz, 1H), 8.05 (dd, 8.16, 7.02 Hz, 1H) and 4.12 (s, 3H)
^{13}C NMR (75 MHz, $CDCl_3$, δ)	166.07, 165.25, 146.93, 143.25, 139.67, 139.02, 137.72, 135.45, 132.74, 131.78, 131.22, 130.57, 129.16, 124.34 and 52.66

[a] Ethyl acetate; [b] 4 × (30 + 250) mm ODS-Hypersil (10 μ); Eluant: a gradient of water to acetonitrile in 30 minutes; Detection: 220 nm; Flow rate: 2 ml/minute.

Table 2. ^{1}H and ^{13}C NMR spectral data of phencomycin methyl ester (**2**), 1,6-dicarbomethoxy phenazine and 1,9-dicarbomethoxy phenazine.

Position	δ_C (75 MHz, $CDCl_3$)			δ_H (300 MHz, $CDCl_3$)
	2	1,6-	1,9-	**2**
1	131.36	131.55	132.40	—
2	134.31	134.35	133.20	8.32 (dd, J=8.16, 1.22 Hz)
3	129.59	129.60	130.00	7.31 (dd, J=8.16, 7.22 Hz)
4	132.93	132.90	132.60	8.50 (dd, J=7.22, 1.22 Hz)
4a	143.04	143.15	142.75	—
5a	143.04	143.15	142.75	—
6	131.36	131.55	132.60	—
7	134.31	134.35	130.00	8.32 (dd, J=8.16, 1.22 Hz)
8	129.59	129.60	133.20	7.31 (dd, J=8.16, 7.22 Hz)
9	132.93	132.90	132.40	8.50 (dd, J=7.22, 1.22 Hz)
9a	143.04	143.15	141.10	—
10a	143.04	143.15	141.10	—
OCH_3	52.74	52.70	52.60	4.15 (s, 6H)
CO	166.90	166.95	167.25	—

Table 3. Antibacterial activity (MIC) of phencomycin (**1**).

Test organism	MIC (μg/ml)
Staphylococcus aureus 209P	100
Staphylococcus aureus 20424	>100
Staphylococcus aureus 3066	>100
Staphylococcus epidermidis 825	>100
Bacillus subtilis	>100
Streptococcus faecalis ATCC 29212	>100

The physico-chemical properties of **1** are listed in Table 1. The IR and ^{1}H NMR spectral data of phencomycin (**1**) gave an early indication for the presence of a carbomethoxy group (IR: 1720 cm^{-1}; δ_H: 4.12) and a carboxyl group (IR: 3450 and 1700 cm^{-1}; δ_H: 15.11 (D_2O exchangeable)). The UV absorption bands at 254 and 368 nm suggested that it belonged to phenazine class of compounds. Thus, phencomycin is a phenazine having carboxyl and carbomethoxy groups as the two substituents. The presence of two *ortho* coupled protons (δ 8.08 and 8.05) and four *ortho* and *meta* coupled protons (δ 9.02, 8.63, 8.44 and 8.39) in the ^{1}H NMR spectrum of **1** suggested that the two substituents could be present at 1 and 6 or 1 and 9 positions.

Phencomycin (**1**) on methylation using CH_2N_2/ether-CH_2Cl_2 mixture at 0°C for 1 hour followed by preparative TLC on silica gel (Article No. 13794, E. Merck) using ethyl acetate as the solvent afforded a methyl ester (**2**) as yellow crystalline solid. The ^{1}H and ^{13}C NMR spectral data of **2** are summarized in Table 2. A comparison of the ^{13}C NMR data of **2** with that of 1,6- and 1,9-dicarbomethoxy phenazine[3] revealed that **2** was identical to 1,6-dicarbomethoxy phenazine. Thus, the structure of phencomycin is established as represented by **1**. During the course of this work we came across a report[4] describing the synthesis of 2,3,4,7,8,9-d_6-phenazine-1,6-dicarboxylic acid monomethyl ester from the corresponding dicarboxylic acid.

Biological Properties

Phencomycin (**1**) exhibited weak antibacterial activity in *in vitro* against Gram +ve bacteria and did not exhibit any antifungal activity. The MIC values of **1** are given in Table 3. Further, phencomycin also exhibited weak inhibition of physiologically important enzyme like renin (IC_{50}: 440 μg/ml).

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