Novel Antibiotics Pyrisulfoxin A and B Produced by *Streptomyces californicus*

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In the course of our screening for new antibiotics active against P388 murine leukemia cells, novel antibiotics named pyrisulfoxin A (**1**) and pyrisulfoxin B (**2**) were isolated from the culture broth of *Streptomyces californicus* BS-75. In this paper, we report the isolation and structures of **1** and **2**.

The producing organism was cultivated at 27°C for 4 days on a rotary shaker in 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting of dextrin 2%, galactose 2%, corn steep liquor 0.5%, bacto soytone 1%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2%. The pH of the medium was adjusted to 7.4 before sterilization.

After removal of the mycelium by centrifugation, the supernatant (8 liters) was adjusted to pH 7.0 with 1 N HCl and applied to a column of Dowex 1 (OH^-). The column was washed with water and then the active principle was eluted with 0.5 N HCl. The eluate was adjusted to pH 7.0 with 3 N NaOH, and applied to a column of Diaion HP-20. The active principle was eluted with MeOH and after evaporation, the residue was applied to an ODS column. The active fraction eluted with 70% aqueous MeOH was finally subjected to preparative HPLC using a YMC ODS column developed with 40% aqueous CH_3CN. Active eluates were separately concentrated to dryness to yield colorless powders of **1** (6.5 mg) and **2** (1.6 mg).

The physico-chemical properties of **1** and **2** are shown in Table 1. The molecular formula of **1** was determined as $C_{13}H_{13}N_3O_3S$ by HRFAB-MS. As summarized in Table 2, the ^{13}C and ^1H NMR spectral data of **1** are very similar to those of caerulomycin A (**3**)[1,2] which was isolated as an antimicrobial substance.

Comparison of ^{13}C NMR spectral data of these two compounds enabled us to assign 11 out of the 13 signals of **1**. The spectral differences between **1** and **3** were that the olefinic methine observed at δ_C 105.5 in **3** was replaced by a quaternary olefinic carbon at δ_C 127.7 (C-5) in **1** with appearance of a new methyl singlet (C-9) at δ_C 39.4 in the latter. From the molecular formula of **1** and the chemical shifts of C-5 and C-9, this new methyl group was ascribed to a methylsulfoxide group. The HMBC spectrum of **1** showed a long-range coupling from the methyl signal (9-H) to C-5 (Fig. 2). Therefore, the methylsulfoxide group was allocated at the C-5 position. The configuration of the oxime group was determined to be *anti* based on a weak NOE observed between 7-H

Table 1. Physico-chemical properties of pyrisulfoxin A and pyrisulfoxin B.

	Pyrisulfoxin A	Pyrisulfoxin B
Appearance	Colorless powder	Colorless powder
MP(℃)	178~180	163~165
Molecular formula	$C_{13}H_{13}N_3O_3S$	$C_{13}H_{11}N_3O_2S$
HRFAB-MS		
Calcd	229.0757 $(M+H)^+$	274.0652 $(M+H)^+$
Found	229.0787	274.0673
UV λ_{max}^{MeOH} nm (ε)	243 (23,500)	243 (17,900)
	288 (14,500)	288 (12,700)
IRυ_{max}(KBr) cm^{-1}	3180, 1565, 1540, 1465, 1355, 990, 730	2230, 1570, 1540, 1460, 1370, 1250, 955, 750

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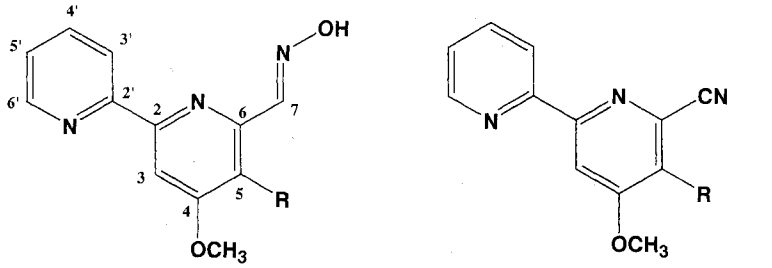
Table 2. ^{13}C and ^{1}H NMR of pyrisulfoxin A (**1**), pyrisulfoxin B (**2**) and caerulomycin A (**3**).

	1*		2*		3**	
No.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	159.7		161.2		156.9	
3	104.3	8.12 (s)	106.6	8.29 (s)	106.5	7.91
4	165.6		164.7		166.6	
5	127.7		131.9		105.5	7.35
6	150.6		137.4		153.4	
7	147.7	8.88 (s)	114.5		148.8	8.72
8	56.6	4.13 (s)	57.0	4.15 (s)	55.5	3.96
9	39.4	3.06 (s)	39.9	3.09 (s)		
2'	154.4		152.9		154.6	
3'	122.0	8.50 (ddd 8.0, 1.3, 1.0)	122.2	8.51 (ddd 8.0, 1.0, 1.0)	120.7	8.40
4'	137.2	7.83 (ddd 8.0, 7.5, 1.8)	137.4	7.87 (ddd 8.0, 7.5, 1.8)	137.2	7.98
5'	124.9	7.39 (ddd 7.5, 4.9, 1.1)	125.5	7.40 (ddd 7.5, 4.9, 1.1)	124.4	7.51
6'	149.1	8.68 (ddd 4.8, 1.8, 1.0)	149.2	8.67 (ddd 4.8, 1.8, 1.0)	149.2	8.72
NOH		9.16 (b)				11.72

* Taken in $CDCl_3$

** Data taken from ref. 3

Fig. 1. Structures of pyrisulfoxin A (**1**) and B (**2**).



R = SCH$_3$ Pyrisulfoxin A (1)
R = H Caerulomycin A
R = SCH$_3$ Collismycin A, SF2738A

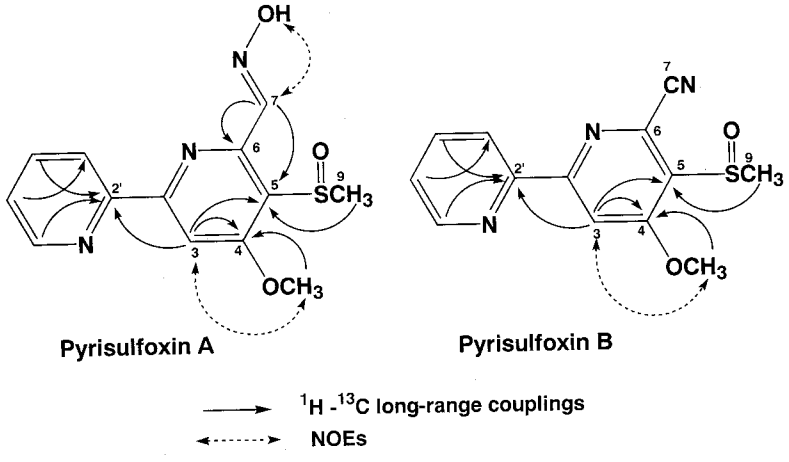
R = SCH$_3$ Pyrisulfoxin B (2)
R = H Caerulomycin nitril
R = SCH$_3$ Collismycin B, SF2738D

and N–OH. These data suggested that the structure of **1** is as shown in Fig. 1.

The molecular formula of **2** was determined as $C_{13}H_{11}N_3O_2S$ by HRFAB-MS. The IR spectrum showed a weak absorption band at 2230 cm^{-1} characteristic to a cyano group and no hydroxyl absorption. Comparison of the NMR data of **2** with those of **1** showed the absence of the protons due to the oxime group (H-7, N–OH), and an upfild shift of C-7 (δ_C 114.5 *vs.* δ_C 147.7). From these results, the structure of **2** was deduced as shown in Fig. 1.

1 showed cytotoxicity against P388 murine leukemia cells with IC_{50} values of 0.1 μg/ml. However, **2** showed no cytotoxicity against P388 murine leukemia cells. Re-

Fig. 2. ^{1}H-^{13}C long-range couplings and NOEs.



cently, collismycin A and SF2738A have been reported as antimicrobial and antitumor agents.[3,4] In place of the methyl sulfoxide function in **1**, these compounds possess a methylthio group at the 5 position of **1**. The cytotoxic activity of **1** was comparable to that of collismycin A and SF2738A. Detailed studies on other biological activities of pyrisulfoxins are under way.

Acknowledgments

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