Communication to the Editor

COLLISMYCINS A AND B, NOVEL NON-STEROIDAL INHIBITORS OF DEXAMETHASONE-GLUCOCORTICOID RECEPTOR BINDING

Sir:

Glucocorticoids are very important anti-inflammatory agents in clinical use[1], but some side effects such as pituitary-adrenal suppression and fluid and electrolyte disturbances are known[2]. In the screening course for new non-steroidal glucocorticoide-like anti-inflammatory agents using the dexamethasone-glucocorticoid receptor binding assay[3], we have found novel compounds named collismycin A and B from a culture of *Streptomyces* sp. MQ22, which was isolated from a soil sample collected at Nirasaki-shi, Nagano prefectutre, Japan. In this communication, we report on the fermentation, isolation, structure elucidation and biological activities of collismycins A and B.

The fermentation was carried out in 500-ml Erlenmeyer flasks containing 100 ml of medium with following composition: potato starch 3.0%, soya flake 1.5%, yeast extract 0.2%, corn steap liquir 0.5%, NaCl 0.3%, $CaCO_3$ 0.3%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CoCl_2 \cdot 6H_2O$ 0.0005%. The pH of the medium was adjusted to 7.1 before sterilization. The culture was carried out at 27°C for 5 days on a rotary shaker.

Collismycins A (**1**) and B (**2**) were isolated according to the scheme as shown in Fig. 2, and showed the physico-chemical properties as summarized in Table 1.

The molecular formula of **1** was determined to be $C_{13}H_{13}N_3O_2S$ by HRFAB-MS data. Since the UV and IR absorption spectra of **1** are very similar to those of caerulomycin A (**3**)[4~6] which was isolated as an antimicrobial agent, it is suggested that **1** has the 2,2'-dipyridyl moiety.

The ^1H and ^{13}C NMR spectra of **1** showed close similarity to those of **3** (Table 2). While the sp^2 methine signal at 5 in **3** was disappeared, a singlet methyl signal (9-H, δ_H 2.39, C-9, δ_C 18.5) was observed in **1**. In the HMBC experiment on **1**, the long range coupling from the methyl signal (9-H) was observed only to the C-5 (δ_C 122.1) (Fig. 3). Taking into consideration the presence of one sulfur atom in the molecular formula of **1** and the chemical shifts of 9-H, C-5 and C-9, SCH_3 group was confirmed to be located at C-5 position. From these findings, the structure of **1** was deduced as shown in Fig. 1.

The molecular formula of **2** was determined to be

Fig. 1. Total structures of collismycins A and B.

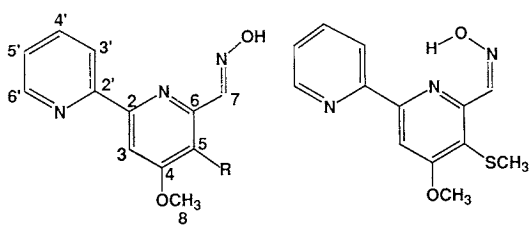

Fig. 2. Isolation scheme of collismycins A and B.

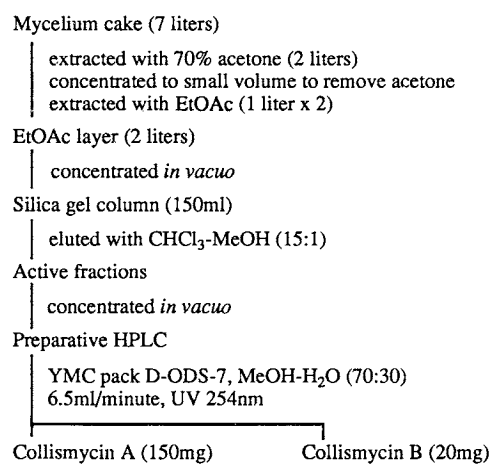

Table 1. Physico-chemical properties of collismycins A and B.

	Collismycin A	Collismycin B
Appearance	Colorless powder	Colorless powder
MP (°C, dec)	170~172	148~150
Molecular formula	$C_{13}H_{13}N_3O_2S$	$C_{13}H_{13}N_3O_2S$
HRFAB-MS		
Calcd:	276.0807	276.0807
Found:	276.0796	276.0798
UV λ_{max} (ε)	243 (26,400)	245 (20,800)
IR ν (KBr) cm^{-1}	3155, 1568, 1540, 1367, 1344, 1215, 995, 794	2937, 1587, 1578, 1544, 1471, 1375, 1064, 962, 904, 792

Table 2. ^{13}C NMR and ^{1}H NMR spectral data of collismycins A (**1**)[a], B (**2**)[a] and caerulomycin A (**3**)[b].

Position	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	157.4 s		154.8 s		156.9 s	
3	103.7 d	8.05 (s)	104.9 d	8.12 (s)	106.5 d	7.91
4	167.4 s		168.2 s		166.6 s	
5	122.1 s		121.0 s		105.5 d	7.35
6	152.6 s		152.5 s		153.4 s	
7	147.6 d	9.10 (s)	140.2 d	8.67 (s)	148.8 d	8.22
8	56.4 q	4.12 (s)	56.7 q	4.18 (s)	55.5 q	3.96
9	18.5 q	2.39 (s)	18.4 s	2.41 (s)		
NOH		10.19 (br s)		16.70 (s)		11.72
2′	155.2 s		153.1 s		154.6 s	
3′	121.9 d	8.55 (d 8.0)	121.0 d	8.10 (d 8.2, 1.0, 1.0)	120.7 d	8.40
4′	137.2 d	7.88 (ddd 8.0, 7.5, 1.2)	137.5 d	7.85 (ddd 8.4, 8.2, 1.8)	137.2 d	7.98
5′	124.3 d	7.34 (dd 7.5, 5.0)	124.9 d	7.38 (dd 8.4, 5.0, 1.0)	124.4 d	7.51
6′	148.9 d	8.67 (dd 5.0, 1.2)	149.5 d	8.68 (dd 5.0, 1.8, 1.0)	149.2 d	8.72

[a] Taken in $CDCl_3$.
[b] Taken in DMSO-d_6.

Fig. 3. ^{1}H-^{13}C long range couplings and NOE.

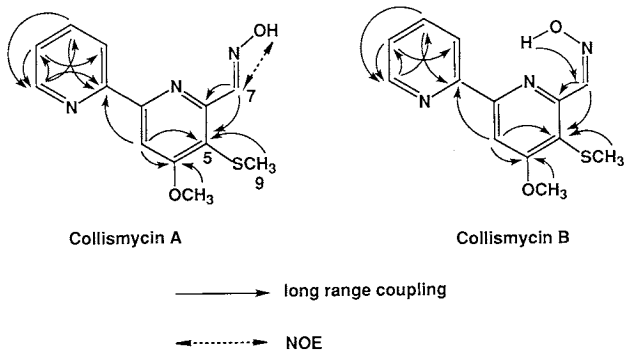

Table 3. Antimicrobial activities of collismycins A (**1**) and B (**2**).

	Medium[a]	Diameter of inhibition zone (mm)	
		1	2
Staphylococcus aureus FDA 209P JC-1	I	14	9.5
Bacillus subtilis ATCC 6633	I	13.5	0
E. coli NIHJ-JC-2	I	(22)	0
Candida albicans Yu1200	II	17	12
Saccharomyces cerevisiae ATCC 9763	II	14	0
Aspergillus niger ATCC 9642	II	23	17

Number in parenthesis indicates a faint inhibition zone.
Plate diffusion assay. 50 μg was applied onto 8 mm filter disk. The disks were placed on plates seeded with the tested microorganisms in the top of the agar.
[a] I: Nutrient agar (Difco), II: SABOURAUD dextrose agar (Difco).

the same as that of **1** by the HRFAB-MS data. Comparison of ^{13}C NMR signals of **2** with those of **1** (Table 2) revealed an upfield shift for C-7 (δ_C 140.2 *vs.* δ_C 147.6), indicating that they were geometrical isomers of the oxime function (**1** for *anti* and **2** for *syn*). This was confirmed because Nuclear

Overhauser effect (NOE) was observed between 7-H and NOH with **1**, but not with **2** (Fig. 3). And thus, the structure of **2** was determined (Fig. 1).

Tested so far, **1** and **2** were not interchangeable in any organic solvents at room temperature. By heating in *ortho*-dichlorobenzene at 120°C under algon atmosphere, **1** was gradually changed to **2**.

The assay of dexamethasone-glucocorticoid receptor binding was examined using rat river cytosol as described previously[3]. **1** and **2** inhibited dexamethasone-glucocorticoid receptor binding in a dose dependent manner, with IC_{50} values of 1.5×10^{-5} M and 1.0×10^{-5} M, respectively. Further biological studies were in progress, and will be reported in the future.

Recently, **1** was reported as antimicrobial and antitumor agent by GOMI *et al.*[7]. Therefore, these activities of **1** and **2** were tested. The results of antimicrobial activities were summarized in Table 3. **1** and **2** showed cytotoxicity against L1210 murine leukemia cells (IC_{50} 0.08 μg/ml and 0.12 μg/ml, respectively).

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References

1) HINCH, P. S.; C. H. SLOCUMB, A. R. BARNES, H. L. SMITH, H. L. POLLEY & E. C. KENDALL: The effects of adrenal cortical hormone 17-hydroxy-11-dehydrocorticosterone (compound E) on the acute phase of rheumatic fever. Mayo Clin. Proc. 24: 277, 1949
2) GILMAN, G. A.; L. S. DOODMAN, T. W. RALL & F. MURAD: GOODMAN and GILMAN's The Pharmacological Basis of Therapeutics. 7th Ed., pp. 1459~1489, Macmillan Publishing Company, a Division of Macmillan, Inc., 1985
3) BAXTER J. D.; D. V. SANTI & G. G. ROUSSEAU: A filer technique for measurement of steroid-receptor binding. Methods Enzymol. 36: 234~239, 1975
4) FUNK, A. & P. V. DIVEKAR: Caerulomycin, a new antibiotic from *Streptomyces caeruleus* Baldacci. I. Production, isolation, assay and biological properties. Can. J. Microbiol. 5 : 317~321, 1959
5) DIVEKAR, P. V.; G. READ & L. C. VINING: Caerulomycin, a new antibiotic from *Streptomyces caeruleus* Balacci. II. Structure. Can. J. Chem. 45: 1215~1223, 1967
6) MCINNES, A. G.; D. G. SMITH, J. L. C. WRIGHT & L. C. VINING: Caerulomycins B and C, new 2,2'-dipyridyl derivatives from Streptomyces caeruleus. Can. J. Chem. 55: 4159~4165, 1977
7) GOMI, S.; S. ITO, K. AZITO, Y. KARIYA & M. KOYAMA (Meiji Seika Kaisya, Ltd.): A new microbial and antitumor agent SF2738. Jpn. KoKai 78322 ('93), Mar. 30, 1993