Communication to the Editor

NOVEL ANTIBIOTICS, COCHLEAMYCINS A AND B

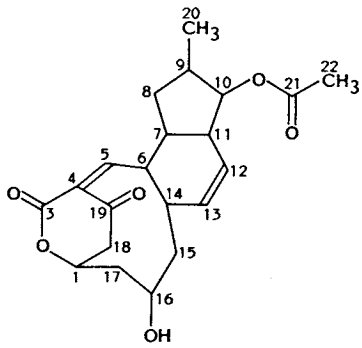
Sir:

In the course of screening for new antitumor antibiotics, we have found novel antibiotics named cochleamycins A and B (Fig. 1) from a culture of *Streptomyces* sp., which was isolated from a soil sample collected at Nishimeya-cho, Aomori, Japan. Cochleamycins A and B were formerly called DT136 A and B[1] and the producing organism has been deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, with an accession No. of FERM BP-2298. In this communication, we report on the fermentation, isolation, structure elucidation and biological activities of cochleamycins A and B.

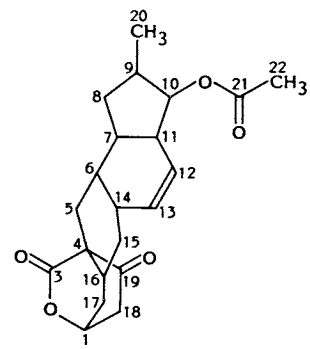
The fermentation was carried out in 500-ml Erlenmeyer flasks containing 100 ml of medium with following composition: glucose 2.5%, soybean meal 1.5%, dry yeast 0.2% and $CaCO_3$ 0.4%. The pH of the medium was adjusted to 7.2 before sterilization. The seed culture was inoculated into the flasks, and the fermentation was carried out at 27°C for 7 days on a rotary shaker.

After removal of the mycelium, the supernatant (4 liters) was applied to a column of Diaion HP-20. The column was washed with 15% MeOH and active materials were eluted with MeOH. The eluate was concentrated *in vacuo* to a minimum volume and extracted with EtOAc at pH 7. The extract was evaporated to dryness and subjected to silica gel column chromatography (Wakogel C-200) using $CHCl_3$-MeOH (100:1). In this chromatography, cochleamycins A (**1**) and B (**2**) were separated from each other. The fraction of **1** was subjected to Sephadex LH-20 chromatography (solvent; MeOH) to give pure **1** (22 mg). The fraction of **2** was developed on a centrifugal partition chromatograph using the solvent system of hexane-MeOH (2:1), and 7 mg of pure **2** was obtained. The physicochemical properties of **1** and **2** are summarized in

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



Cochleamycin A



Cochleamycin B

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

	Cochleamycin A	Cochleamycin B
Appearance	Colorless powder	Colorless powder
MP (°C, dec)	200~203	133~135
$[\alpha]_D^{25}$	+107° (*c* 1.0, MeOH)	+83° (*c* 0.1, MeOH)
Molecular formula	$C_{21}H_{26}O_6$	$C_{21}H_{26}O_5$
HRFAB-MS Calcd:	375.1906	359.1802
Found:	375.1857 $(M+H)^+$	359.1830 $(M+H)^+$
UV λ_{max}^{MeOH} $(E_{1cm}^{1\%})$	245 (146)	End absorption
IR ν (KBr) cm^{-1}	3450, 2950, 1750, 1715, 1710, 1240	2950, 1760, 1740, 1735, 1240

Table 2. 125 MHz ^{13}C NMR and 500 MHz ^{1}H NMR spectral data of cochleamycins A and B[a].

Position	Cochleamycin A		Cochleamycin B	
	δ_C	δ_H	δ_C	δ_H
1	72.8 d	4.98 (ddd 2.4, 4.5, 7.5)[b]	73.0 d	4.98 (m)
3	165.7 s		169.9 s	
4	136.1 s		60.4 s	
5	154.0 d	6.77 (d 11.3)	24.7 t	1.65[c], 1.82[c]
6	40.6 d	3.30 (ddd 2.4, 5.6, 11.3)	31.9 d	2.25 (m)
7	35.6 d	2.72 (ddd 2.4, 6.2, 17.0)	39.4 d	2.47[c]
8	34.9 t	1.68~1.70[c]	35.5 t	1.59~1.63[c]
9	35.5 d	2.05[c]	35.5 d	2.15 (m)
10	82.2 d	4.94 (d 3.8)	82.6 d	4.88 (d 4.0)
11	42.5 d	2.84 (m)	42.8 d	2.64 (m)
12	128.6 d	5.68 (ddd 3.2, 3.2, 10.5)	130.2 d	5.37 (ddd 2.0, 3.2, 10.0)
13	128.6 d	5.91 (ddd 2.4, 2.4, 10.5)	129.3 d	5.62 (ddd 3.0, 3.0, 10.0)
14	34.5 d	2.92 (m)	29.0 d	2.47[c]
15	42.5 t	1.69[c], 1.97 (ddd 6.8, 6.8, 16.8)	38.2 t	1.46 (ddd 4.5, 6.8, 14.0), 1.90 (ddd 3.5, 3.5, 14.0)
16	66.5 d	3.62 (ddd 2.4, 6.8, 11.8)	30.3 d	2.05[c]
17	45.6 t	1.78 (ddd 2.4, 2.4, 15.5), 2.48 (ddd 4.5, 11.8, 15.5)	33.3 t	1.82[c], 2.18 (ddd 1.5, 10.0, 14.5)
18	41.1 t	2.66 (d 19.0), 3.11 (dd 7.5, 19.0)	41.3 t	2.42 (dd 1.5, 19.0), 2.72 (ddd 3.2, 3.2, 19.0)
19	194.2 s		203.1 s	
20	13.9 q	0.92 (d 7.0)	14.1 q	0.89 (d 6.8)
21	170.9 s		170.8 s	
22	21.1 q	2.08 (s)	21.1 q	2.06 (s)

[a] Taken in $CDCl_3$.
[b] Coupling constants in J=Hz.
[c] Resonance in one-dimensional spectra obscured by overlapping signals.

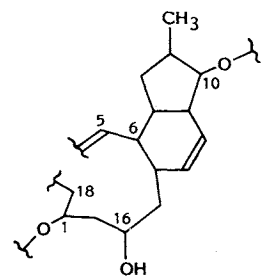
Table 1.

The molecular formula of **1** was determined to be $C_{21}H_{26}O_6$ by HRFAB-MS data. IR absorptions (1750, 1715 and 1710 cm^{-1}) and ^{13}C NMR signals (δ_C 165.7, 170.9 and 194.2) of **1** indicated the presence of two ester and one ketone groups. IR absorption at 3450 cm^{-1} also proved the existence of a hydroxyl group in **1**.

The partial structure of **1** shown in Fig. 2 was determined by the analysis of ^{1}H-^{1}H COSY and decoupling experiments. These experiments and ^{13}C-^{1}H COSY spectrum confirmed that the hydroxyl group was attached to C-16 (δ_H 3.62, δ_C 66.5). Further structural elucidation was performed by the observation of the long range ^{1}H-^{13}C connectivities which were detected by heteronuclear multiple-bond correlation (HMBC)[2] and long range selective proton decoupling (LSPD) experiments.

As shown in Fig. 3, the HMBC experiment on **1** showed the long range couplings of 10-H (δ_H 4.94) and 22-H (δ_H 2.08) to C-21 (δ_C 170.9). Thus, the

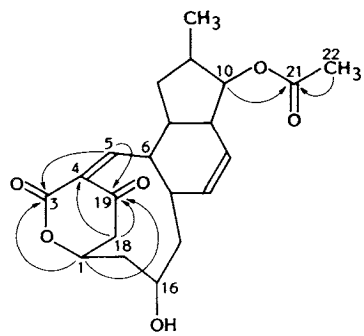
Fig. 2. Partial structure of cochleamycin A.



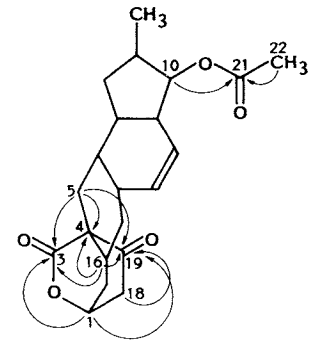
acetoxy side chain was determined to attach to C-10. The long range couplings of 5-H (δ_H 6.77) to C-3 (δ_C 165.7) and C-19 (δ_C 194.2), and 18-H (δ_H 2.66, 3.11) to C-4 (δ_C 136.1) and C-19 were also observed by the HMBC experiment. Therefore, the connectivities of C-5–C-4–C-3 and C-5–C-4–C-19–C-18 were confirmed. Furthermore, the LSPD experiment on **1** showed the linkage from C-1 to C-3 through the oxygen atom. Thus, we could establish the (α,β-unsaturated) β-keto δ lactone ring, and

Fig. 3. Long range couplings observed by HMBC and LSPD experiments.

⟶ Long range coupling.



Cochleamycin A



Cochleamycin B

Table 3. Antimicrobial activity of cochleamycin A.

Organism	Medium[a]	Diameter of inhibition zone (mm)
Staphylococcus aureus FDA 209P	I	16
S. aureus MS14146[b]	I	11
S. aureus MS14287[b]	I	15
Micrococcus luteus ATCC 9341	I	25
Bacillus subtilis ATCC 6633	I	15
B. subtilis PCI 219	I	15
Escherichia coli NIHJ	I	0
Pseudomonas aeruginosa NCTC 10490	I	0
Candida albicans Yu 1200	II	0
Saccharomyces cerevisiae ATCC 9763	II	0

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).

[b] Methicillin-resistant.

the structure of **1** was determined as shown in Fig. 1.

The molecular formula of **2** was determined to be $C_{21}H_{26}O_5$ by HRFAB-MS. The formula contained one oxygen atom less than that of **1**. ^1H-^1H COSY and decoupling experiments **2** proved the unit from C-6 to C-14 in **1** was completely preserved in the structure of **2**. Comparison of the ^{13}C NMR data of **1** and **2** revealed an upfield shift of C-16 from δ_C 66.5 to 30.3. This shift and the HRFAB-MS data indicated the absence of a hydroxyl group at C-16 in the structure of **2**. Furthermore, olefinic carbon signals of C-4 and C-5 in the structure of **1** were absent in **2**, and in turn, one methylene carbon (δ_C 24.7) and one quarternary carbon (δ_C 60.4) were observed. The ^1H-^1H COSY experiment proved that these methylene protons were coupled to 6-H (δ_H 2.25) and were assigned as 5-H.

In the HMBC spectrum of **2**, the 5-H protons (δ_H 1.65, 1.82) were coupled to the quarternary carbon C-4 (δ_C 60.4), C-3 (δ_C 169.9) and C-19 (δ_C 203.1) (Fig. 3). The oxymethine proton 1-H (δ_H 4.98) also showed couplings to C-3 and C-19. Long range couplings were observed between 16-H (δ_H 2.05) and C-3, C-4 and C-19 by a LSPD experiment. Thus, the preservation of the β keto δ lactone unit in **1** and linking of C-5 and C-16 through C-4 were elucidated, and the structure of **2** was determined (Fig. 1).

Studies of the absolute configurations of **1** and **2** are now in progress, and will be reported in the future.

1 and **2** showed cytotoxicity against P388 leukemia cells (IC_{50} 1.2 μg/ml and 2.6 μg/ml, respectively). **1** showed weak antimicrobial activity against Gram-positive bacteria as shown in Table 3.

Acknowledgment

We thank Dr. M. INOUE of the Laboratory of Drug Resistance in Bacteria, School of Medicine, Gunma University for strains of methicillin-resistant *Staphylococcus aureus* MS14146 and MS14287.

KAZUTOSHI SHINDO
HIROYUKI KAWAI

Pharmaceutical Research Laboratory,
Kirin Brewery Co., Ltd.,
Takasaki, Gunma 370-12, Japan

(Received September 5, 1991)

References

1) SHINDO, K.; H. KAWAI & M. MATSUOKA (Kirin Brewery): New substances DT136 A and B. Jpn. Kokai 286683 ('90), Nov. 26, 1990

2) BAX, A. & M. F. SUMMERS: ^{1}H and ^{13}C assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. J. Am. Chem. Soc. 108: 2093~2094, 1986