Studies on Cochleamycins, Novel Antitumor Antibiotics

II. Physico-chemical Properties and Structure Determination

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The structure of cochleamycins A, A2, B and B2 (Fig. 1), novel antitumor antibiotics, were elucidated by NMR spectral analysis. Cochleamycins were found to possess novel carbocyclic skeletons.

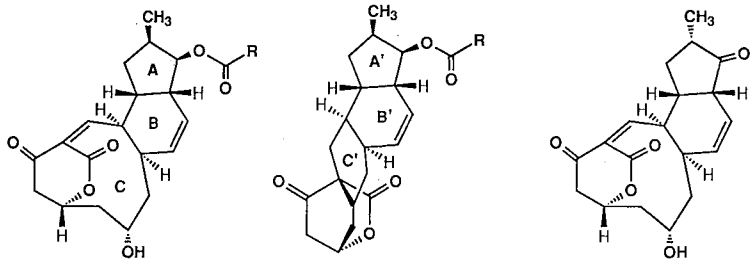
In the previous paper, we described the taxonomy of the producing microorganism, the production, the isolation, and biological activities of cochleamycins[1]. This paper describes the physico-chemical properties and structural studies on cochleamycins.

Cochleamycin A (**1**) is a colorless powder with properties listed in Table 1. The molecular formula of **1** was determined to be $C_{21}H_{26}O_6$ by HRFAB-MS. IR absorptions at 3450, 1750, 1715 and 1710 cm^{-1} showed the existence of a hydroxyl group and three carbonyl functions in **1**.

The ^1H NMR spectrum of **1** taken in $CDCl_3$ (Fig. 2) revealed the presence of two methyl resonances (δ_H 0.92 and δ_H 2.08), three olefin methine protons (δ_H 5.63, 5.91 and 6.77) in addition to 16 other methylene methine protons at around δ_H 1.68~4.94. The ^{13}C NMR spectrum of **1** showed 21 carbon signals. The DEPT spectra indicated the presence of two methyls, four methylenes, eleven methines and four quarternary carbons. The ^{13}C signals of δ_C 165.7, 170.9 and 194.2 showed the presences of two ester and one ketone groups in **1**. The ^1H and ^{13}C spectral data for **1** are summarized in Tables 2 and 3, respectively.

Detailed analysis of the ^1H-^1H and ^{13}C-^1H COSY of **1** proved the partial structures as shown in Fig. 3. These experiments also 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

Fig. 1. Total structure of cochleamycins and macquarimycin A.



R=CH_3 Cochleamycin A (1)
R=$CH(CH_3)_2$ Cochleamycin A2 (2)

R=CH_3 Cochleamycin B (3)
R=$CH(CH_3)_2$ Cochleamycin B2 (4)

Macquarimicin A (5)

Table 1. Physico-chemical properties of cochleamycins.

	A	A2	B	B2
m.p. (℃,dec.)	200 - 203	214 - 216	133 - 135	152 - 154
$[\alpha]_D^{24}$(MeOH)	+107 (c 1.0)	+76 (c 1.0)	+83 (c 0.1)	+104 (c 0.1)
Molecular formula	$C_{21}H_{26}O_6$	$C_{23}H_{30}O_6$	$C_{21}H_{26}O_5$	$C_{23}H_{30}O_5$
HRFAB-MS Calcd:	375.1906	403.2063	359.1802	387.2162
$(M+H)^+$ Found:	375.1857	403.2092	359.1830	387.2148
UV λmax ($E^{1\%}_{1cm}$)	245 (146)	246 (162)	End	End
IR ν (KBr) cm^{-1}	3450, 2950	3513, 2970	2950, 1760	2930, 1761
	1750, 1715	1734, 1709	1740, 1735	1730, 1701

Fig. 2. 500 MHz ^{1}H NMR spectrum of cochleamycin A.

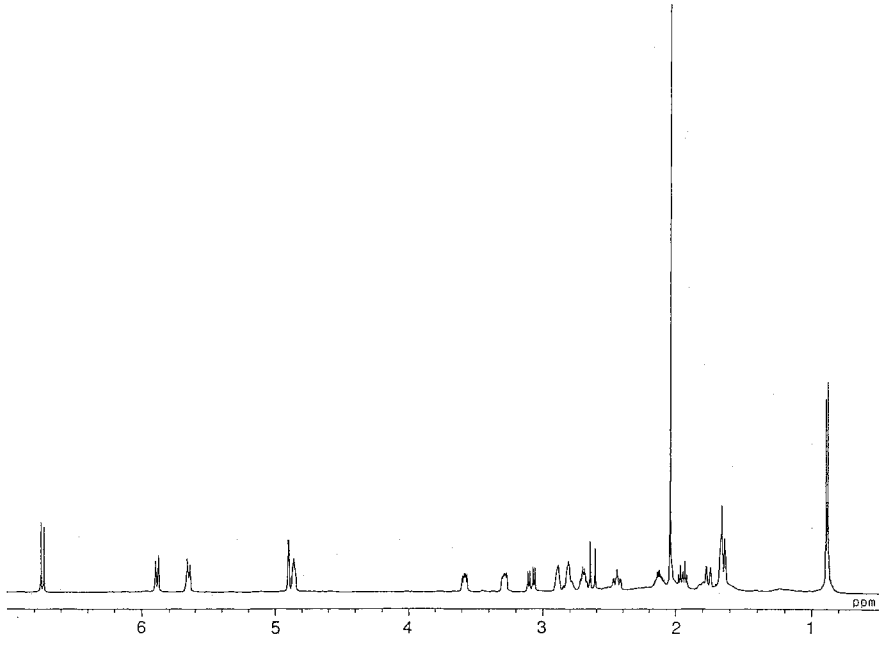


Table 2. 500 MHz ^{1}H NMR spectral data of cochleamycins[a].

Position	A	A2	B	B2
	δ_H	δ_H	δ_H	δ_H
1	4.88 (ddd 2.4, 4.5, 7.5)[b]	4.88 (ddd 3.7, 4.5, 7.5)	4.98 (m)	4.97 (m)
5	6.77 (d 11.3)	6.76 (d 11.5)	1.65[c]	1.65[c]
			1.82[c]	1.82[c]
6	3.30 (ddd 2.4, 5.6, 11.3)	3.30 (ddd 2.4, 5.5, 11.3)	2.25 (m)	2.25 (m)
7	2.72 (dddd 2.4, 7.0, 7.0, 10.0)	2.71 (dddd 2.4, 7.0, 7.0, 10.0)	2.47[c]	2.47[c]
8	1.68[c], 1.70[c]	1.68[c], 1.70[c]	1.59[c], 1.62[c]	1.59[c], 1.62[c]
9	2.05[c]	2.15 (m)	2.15 (m)	2.07 (m)
10	4.94 (d 3.8)	4.90 (d 3.8)	4.88 (d 4.0)	4.86 (d 3.8)
11	2.84 (m)	2.80 (m)	2.64 (m)	2.62 (m)
12	5.63 (ddd 3.0, 3.0, 10.0)	5.67 (ddd 3.2, 3.2, 10.5)	5.62 (ddd 3.0, 3.0, 10.0)	5.63 (ddd 3.0, 3.0, 10.0)
13	5.91 (ddd 2.4, 2.4, 10.0)	5.91 (ddd 2.4, 2.4, 10.5)	5.37 (ddd 2.0, 3.2, 10.0)	5.37 (ddd 2.0, 3.2, 10.0)
14	2.92 (m)	2.91 (m)	2.47[c]	2.47[c]
15	1.69[c]	1.69[c]	1.46 (ddd 4.5, 10.0, 14.0)	1.47 (ddd 4.5, 10.0, 14.0)]
	1.97 (ddd 6.8, 6.8, 16.8)	1.97 (ddd 6.6, 6.6, 16.8)	1.90 (ddd 3.5, 3.5, 14.0)	1.91 (ddd 3.5, 3.5, 14.0)
16	3.62 (ddd 2.4, 6.8, 16.8)	3.60 (ddd 2.4, 6.6, 16.8)	2.05[c]	2.04 (m)
17	1.78 (ddd 2.4, 2.4, 15.5)	1.78 (ddd 2.4, 2.4, 15.5)	1.82[c]	1.82[c]
	2.48 (ddd 4.5, 11.8, 15.5)	2.47 (ddd 4.5, 11.8, 15.5)	2.18 (ddd 1.5, 10.0, 14.5)	2.18 (ddd 1.5, 10.0, 14.5)
18	2.66 (d 19.0)	2.64 (d 19.0)	2.42 (dd 1.5, 19.0)	2.42 (dd 1.5, 19.0)
	3.11 (dd 7.5, 19.0)	3.11 (dd 7.5, 19.0)	2.72 (ddd 3.2, 3.2, 19.0)	2.72 (ddd 3.2, 3.2, 19.0)
20	0.92 (d 7.0)	0.91 (d 7.0)	0.89 (d 6.8)	0.89
22	2.08 (s)	2.55 (qq 7.0, 7.0)	2.06 (s)	2.56 (qq 7.0, 7.0)
23		1.16 (d 7.0)		1.17 (d 7.0)
24		1.17 (d 7.0)		1.18 (d 7.0)

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

range ^{1}H-^{13}C connectivities which were detected by HMBC[2] and LSPD[3] experiments.

As shown in Fig. 4, 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 acetoxy group was determined to be attached 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 confirmed the connectivities of C-5–C-4–C-3 and C-5–C-4–C-19–C-18. Furthermore, the LSPD experiment on **1** showed the linkage from C-1 to C-3 through the oxygen atom. Thus, we could establish the

Table 3. 125 MHz ^{13}C NMR spectral data of cochleamycins[a].

Position	A	A2	B	B2
	δ_C	δ_C	δ_C	δ_C
1	72.8 (d)[b]	72.7 (d)	73.0 (d)	73.0 (d)
3	165.7 (s)	165.7 (s)	169.9 (s)	170.0 (s)
4	136.1 (s)	136.1 (s)	60.4 (s)	60.3 (s)
5	154.0 (d)	156.5 (d)	24.7 (t)	24.6 (t)
6	40.6 (d)	40.9 (d)	31.9 (d)	32.0 (d)
7	35.6 (d)	35.6 (d)	39.4 (d)	39.5 (d)
8	34.9 (t)	35.1 (t)	35.5 (t)	35.5 (t)
9	35.5 (d)	35.6 (d)	35.5 (d)	35.7 (d)
10	82.2 (d)	82.3 (d)	82.6 (d)	82.1 (d)
11	42.5 (d)	42.6 (d)	42.8 (d)	42.8 (d)
12	128.6 (d)	128.5 (d)	129.3 (d)	129.3 (d)
13	128.6 (d)	128.5 (d)	130.2 (d)	130.2 (d)
14	34.5 (d)	34.6 (d)	29.0 (d)	29.0 (d)
15	41.1 (t)	41.1(t)	38.2 (t)	38.2 (t)
16	66.5 (d)	66.5 (d)	30.3 (d)	30.4 (d)
17	45.6 (t)	45.7 (t)	33.3 (t)	33.2 (t)
18	41.1 (t)	41.1 (t)	41.3 (t)	41.3 (t)
19	194.2 (s)	194.2 (s)	203.1 (s)	203.3 (s)
20	13.9 (q)	13.9 (q)	14.1 (q)	14.1 (q)
21	170.9 (s)	176.8 (s)	170.8 (s)	176.8 (s)
22	21.1 (q)	34.3 (d)	21.1 (q)	34.4 (d)
23		19.0 (q)		19.0 (q)
24		19.1 (q)		19.1 (q)

[a] Taken in $CDCl_3$

[b] multiplicities in the off-resonance decoupling; s, singlet; d, doublet; t, triplet; q, quartet.

Fig. 3. Partial structures of cochleamycin A.

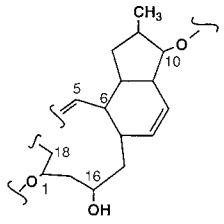
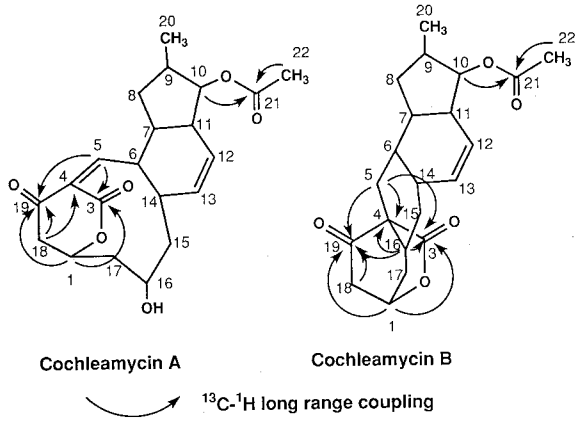


Fig. 4. Long range couplings observed by HMBC and LSPD experiments on cochleamycin A and B.



β-keto δ lactone ring in 1. The geometry of C-4 was determined to be *Z* by the observation of ^{13}C-{^{1}H} NOE between 5-H (δ_H 6.77) and C-19 (δ_C 194.2) as shown in Fig. 5. The structure of **1** which we reported in 1992[4] should be revised. From these findings, the structure of **1** was determined as shown in Fig. 1.

The molecular formula of cochleamycin A2 (**2**) was deduced to $C_{23}H_{30}O_6$ by HRFAB-MS. The ^{1}H NMR spectrum of **2** was quite similar to that of **1**. The singlet methyl (22-H, δ_H 2.05) in **1** was absent, and in turn, two doublet methyls (δ_H 1.16 and 1.17) coupled with a methine (δ_H 2.55) were observed in **2**. Furthermore, the HMBC experiment on **2** showed the long range couplings from the doublet methyls and 10-H (δ_H 4.90) to C-21 (δ_C 176.8). Therefore, it was clarified that the acetoxy group attached at C-10 in **1** was replaced by the isobutyryloxy group in **2**. The structure of **2** was determined as shown in Fig. 1.

The molecular formula of cochleamycin B (**3**) was determined to be $C_{21}H_{26}O_5$ by HRFAB-MS. The molecular contained one oxygen atom less than that of **1**. ^{1}H-^{1}H COSY and decoupling experiments on **3** proved the unit from C-6 to C-14 in **1** was completely preserved in the structure of **3**. Comparison of the ^{13}C NMR data of **1** and **3** revealed an upfield shift of C-16 (methine carbon) from δ_C 66.5 to 30.3. This upfield shift and the HRFAB-MS data indicated the absence of the hydroxyl group at C-16 in the structure of **3**. Furthermore, the olefinic carbon signals of C-4 and C-5 in the structure of **1** were disappeared in **3**, and one methylene carbon (δ_C 24.7) and one quarternary carbon (δ_C 60.4) were observed. The ^{1}H-^{1}H 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 **3**, 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. 4). 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 the LSPD experiment. Thus, it was elucidated that the β keto δ lactone unit in **1** was preserved in the structure of **3** and C-5 was linked to C-16 through C-4. The structure of **3** was determined as shown in Fig. 1.

The molecular formula of cochleamycin B2 (**4**) was determined to be $C_{23}H_{30}O_5$ by HRFAB-MS. Comparison of ^{1}H and ^{13}C NMR data to those of **3** apparently showed that the acetoxy group in **3** was replaced to isobutyryloxy group in **4** as described in the structure

Fig. 5.

a) ^{13}C SINGL-BCM spectrum of cochleamycin A. b) ^{13}C-{^{1}H} NOE difference spectrum of cochleamycin A arising from irradiation of the 5-H at δ_H 6.77.

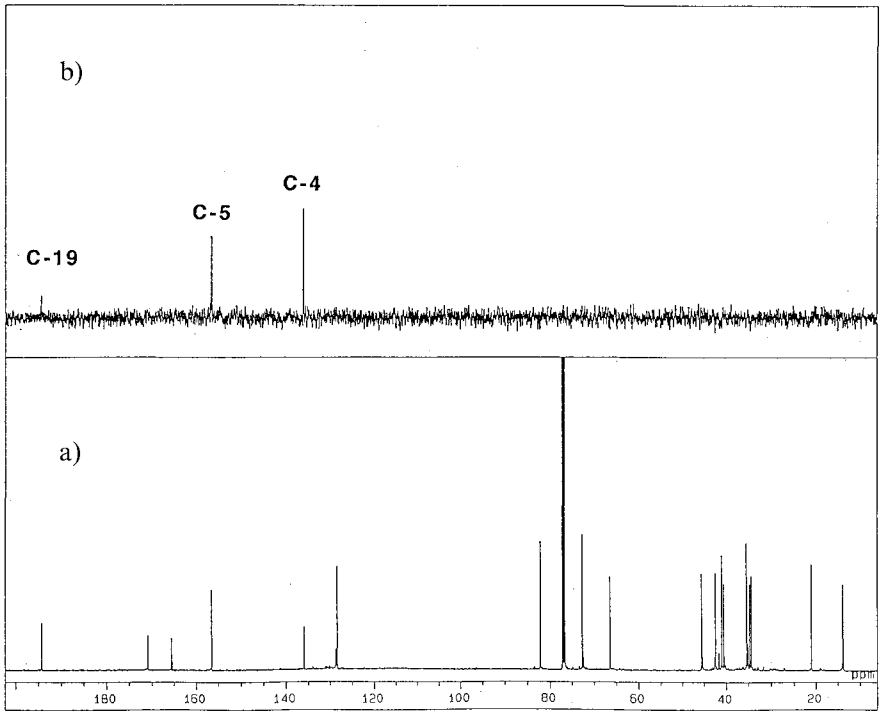


Fig. 6. Phase-sensitive NOESY spectrum of cochleamycin A2.

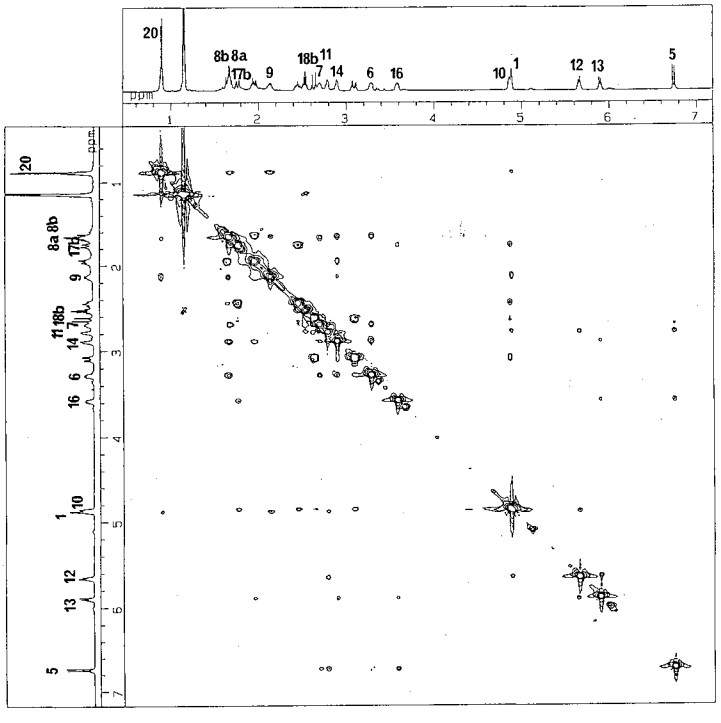


Fig. 7. Relative configuration of cochleamycin A and A2.

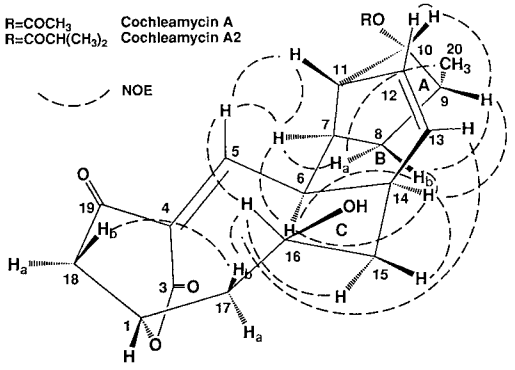
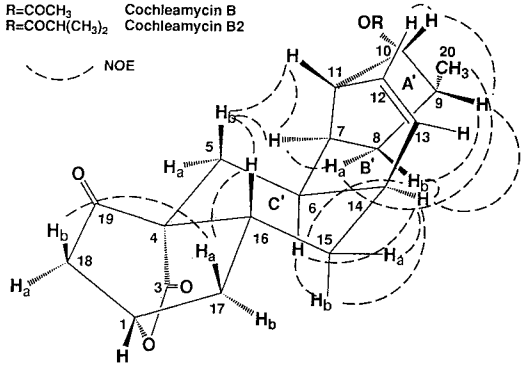


Fig. 8. Relative configuration of cochleamycin B and B2.



elucidation of **2**. And the structure of **4** was confirmed as shown in Fig. 1.

The relative configuration of cochleamycins were determined by the NOESY experiments (Fig. 6) and the *J* values. NOEs observed in these experiments are summarized in Fig. 7 (**1** and **2**) and Fig. 8 (**3** and **4**). The coupling constant of $J_{7,11}$ (7.0 Hz) supported by the NOEs among 5-H, 7-H and 11-H confirmed the AB and A′B′ ring junction to be *cis*. The BC and B′C′ ring junction was also proved to be *cis* by the NOE network in 6-H, 8-Hb (δ_H 1.68 in **2**, δ_H 1.62 in **4**) and 14-H. The relative stereochemistry of the cyclopentane ring (A and A′ rings) was determined by the NOEs observed between 7-H and 8Ha (δ_H 1.70 in 2, δ_H 1.59 in 4), 8Ha and 20-H, 8Hb and 9-H, 9-H and 10-H, 9-H and 14-H and 10-H and 12-H. The relative stereochemistry of the cyclohexane ring (C′ ring) in **3** and **4** was elucidated from the observed NOEs between 5-H and 16-H, and 6-H and 11-H and the large coupling constants of $J_{5ax,6}$ (13.1 Hz) and $J_{15ax,16}$ (13.4 Hz). From the results described above, the relative configurations of cochleamycins were determined as shown in Fig. 1.

Recently, the closely related antibiotics, macquarimicins were reported by JACKSON *et al.*[5,6]. The structure of macquarimicin A (**5**) is shown in Fig. 1. The relative stereochemistry of **5** is almost identical with that of **1** except the C-9 position in the cyclopentane ring. The numbering system adopted for cochleamycins is different from that for macquarimicins. It is to accord with our previous numbering system reported in 1992[4].

Experimental

General

Specific rotation was obtained on a Jusco DIP-140 spectropolarimeter. Mass spectra were measured on JEOL JMS-SX102 A 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-GX500 and α-500 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.

Acknowledgments

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References

1) SHINDO, K.; M. MATSUOKA & H. KAWAI: Studies on cochleamycins, novel antitumor antibiotics. I. Taxonomy, production, isolation and biological activities. J. Antibiotics 49: 241～243, 1996
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
3) SETO, H.; T. SASAKI, H. YONEHARA & J. UMEZAWA: Studies on the biosynthesis of pentalenolactone. Part I. Application of long range selective proton decoupling (LSPD) and selective ^{13}C-{^{1}H} NOE in the structural elucidation of pentalenolactone G. Tetrahedron Lett., 923, 1978
4) SHINDO, K. & H. KAWAI: Novel antibiotics, cochleamycins A and B. J. Antibiotics 45: 292～295, 1992
5) JACKSON, M.; J. P. KARWOWSKI, R. J. THERIAULT, R. R. RASMUSSEN, D. M. HENSEY, P. E. HUMPHRREY, S. J. SWANSON, G. J. BARLOW, U. PREMACHANDRAN & J. B. MCALPINE: Macquarimicins, microbial metabolites from *Micromonospora* I. Discovery, taxonomy, fermentation and biological properties. J. Antibiotics 48: 462～466, 1995
6) HOCHLOWSKI, E. J.; M. M. MULLALLY, R. HENRY, D. M. WHITTERN & J. B. MCALPINE: Macquarimicins, microbial metabolites from *Micromonospora* II. Isolationand structural elucidation. J. Antibiotics 48: 467～470, 1995