VOL. 52 NO. 9, SEPT. 1999 THE JOURNAL OF ANTIBIOTICS pp. 787 — 796

Decatromicins A and B, New Antibiotics Produced by *Actinomadura* sp. MK73-NF4

II. Structure Determination

ISAO MOMOSE, SEHEI HIROSAWA, HIKARU NAKAMURA, HIROSHI NAGANAWA, HIRONOBU IINUMA, DAISHIRO IKEDA* and TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141-0021, Japan

(Received for publication May 17, 1999)

The structures of decatromicins A and B that strongly inhibit the growth of MRSA were elucidated by the analysis of various NMR experiments. The planar structure was determined by ^{1}H, ^{13}C, COSY, HMQC and HMBC NMR spectra. The relative configuration of aglycone was elucidated by NOESY experiments and the absolute structure was determined by application of the modified Mosher's method. The absolute structure of glycosyl moiety was determined by X-ray analysis of the *O*-(*p*-bromobenzoyl) derivative.

New antibiotics decatromicins A (**1**) and B (**2**) isolated from the culture broth of *Actinomadura* sp. MK73-NF4 have shown potent anti-MRSA activity. In the preceding paper, the taxonomy of the producing strain, production, isolation, physico-chemical properties and biological activities of **1** and **2** have been reported[1]. We describe herein the structure determination of **1** and **2** (Fig. 1) including the absolute configurations.

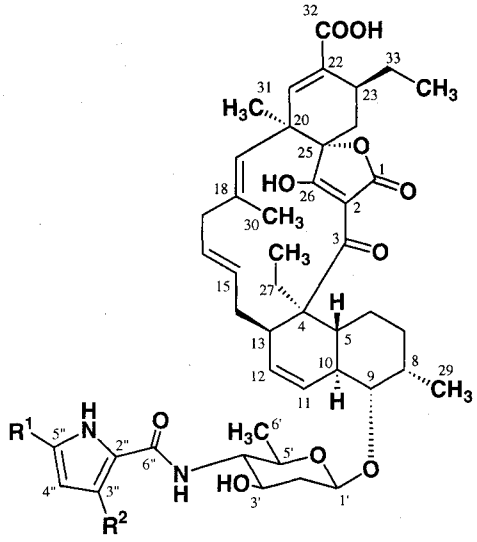
Structure Elucidation of Decatromicin A (**1**)

Decatromicin A (**1**) was obtained as a white powder. The UV spectrum of **1** showed an absorption maximum at 271 nm in MeOH. The absorption band in alkaline solution exhibited characteristic bathochromic shifts. The molecular formula of **1** was established as $C_{45}H_{57}ClN_2O_{10}$ by HRFAB-MS data [found *m/z* 821.3801 $(M+H)^+$, calcd. *m/z* 821.3780 for $C_{45}H_{58}ClN_2O_{10}$] and NMR spectra. ^{1}H and ^{13}C NMR data of **1** are shown in Table 1. The ^{1}H, ^{13}C NMR, DEPT and HMQC spectra of **1** revealed the presence of twenty-seven sp^3 carbons and eighteen sp^2 carbons. The twenty-seven sp^3 carbons consist of six primary, eight secondary, ten tertiary and three quaternary carbon atoms. Out of eighteen sp^2 carbons, ten carbons bear no proton and the others bear one proton each.

The results of ^{1}H-^{1}H COSY and HMBC experiments of **1** are summarized in Fig. 2. ^{1}H and ^{13}C NMR spectra of **1** indicated the presence of a glycopyranosyl moiety in the structure, because the characteristic signals at δ 4.52 (1′-H) and δ 103.00 (C-1′) were assignable to the anomeric signals. The ^{1}H-^{1}H coupling constants of doublet of doublets for 1′-H were 1.6 and 10.0 Hz, which clearly indicated a β-linkage of the sugar. ^{1}H-^{1}H COSY data showed the connectivity from 1′-H to 6′-H (δ 1.20) in the sugar moiety. In HMBC spectrum, the anomeric carbon C-1′ was coupled to 5′-H (δ 3.39). The chemical shifts of 3′-H (δ 3.68) and 4′-H (δ 3.54) suggested that an oxygen atom and a nitrogen atom were attached to C-3′ and C-4′, respectively. In ^{1}H-^{1}H COSY spectrum, an aromatic proton at δ 6.03 (4″-H) showed the connectivity to an aromatic proton at δ 6.79 (5″-H). In HMBC spectrum, both 4″-H and 5″-H were coupled to C-2″ (δ 126.86), C-3″ (δ 119.91) and carbonyl carbon C-6″ (δ 163.24). A long-range correlation between 4′-H and carbonyl carbon C-6″ was observed. These results indicated that a (3-(substituted)pyrrole-2-carbonyl)amino sugar moiety is present in the structure of **1**.

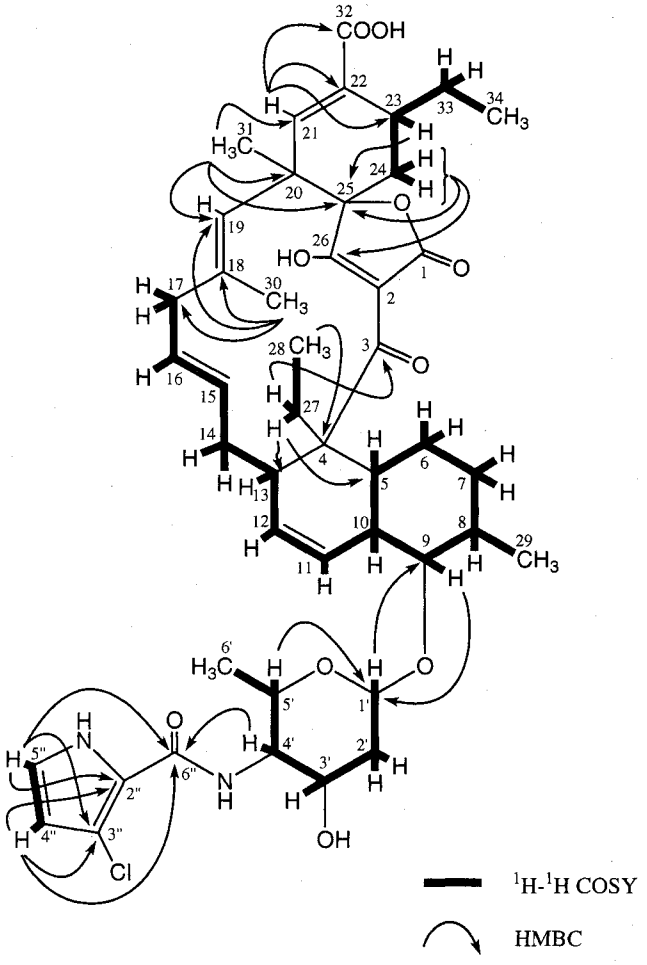
The ^{1}H-^{1}H COSY spectrum revealed connectivity from 5-H (δ 1.84) to 17-H_2 (δ 2.36 and 2.50), from 24-H_2 (δ 1.83 and 2.49) to 34-H (δ 0.92), and from 27-H_2 (δ 1.88

Fig. 1. The structure of decatromicins A (**1**), B (**2**), and derivatives (**11** and **12**).



	R^1	R^2
Decatromicin A (**1**):	H	Cl
Decatromicin B (**2**):	Cl	Cl
11:	Cl	H
12:	H	H

Fig. 2. Summary of ^{1}H-^{1}H COSY and HMBC experiments of **1**.



and 2.73) to 28-H (δ 0.91). In HMBC spectrum, a cross peak between methyl protons at δ 0.91 (28-H) and a quaternary carbon at δ 56.07 (C-4) was observed. Both of C-5 (δ 41.67) and C-13 (δ 44.16) were coupled to 27-H (δ 2.73). The carbon signal at δ 206.02 (C-3) was coupled to 27-H (δ 1.88). These data suggested the presence of an octalin (octahydronaphthalene) ring with an ethyl group and a carbonyl group at C-4. The coupling between 9-H (δ 3.38) of the octalin ring and an anomeric carbon C-1′ revealed 9-*O*-glycosyl structure. Methyl protons at δ 1.78 (30-H) showed cross peaks to C-17 (δ 45.77), C-18 (δ 140.35) and C-19 (δ 126.68). Methyl protons at δ 1.31 (31-H) showed cross peaks to C-19, C-20 (δ 43.85) and C-21 (δ 143.72). An olefin proton 21-H (δ 7.07) showed couplings to C-22 (δ 132.90), C-23 (δ 37.16) and a carboxy carbon C-32 (δ 170.43). The carboxy group could be placed on the C-22 position. A quaternary carbon at δ 87.01 (C-25) adjacent to an oxygen atom was coupled to 31-H, the 23-H (δ 2.71) and 24-H_2 (δ 1.83 and 2.49). The carbon signal at δ 200.14 (C-26) was coupled to 24-H. In ^{13}C NMR spectrum, unassigned carbon signals at δ 169.00 and 104.34 were present. These signals were assigned to be C-1 and C-2 by comparison with other tetronic acid derivatives. Two exchangeable hydroxy protons in ^{1}H NMR spectrum in DMSO-d_6 should be connected to C-3′ (δ 70.26) and C-32 (carboxylic acid) by considering the molecular formula. All signals in ^{1}H and ^{13}C NMR spectra, thus, were assigned, and all correlation found in various experiments of NMR was in perfect agreement with the proposed structure (Fig. 1). Consequently, structure of **1** was found to be a new macrocyclic lactone antibiotic containing a tetronic acid, similar to kijanimicin[2], saccharocarcins[3,4] and pyrrolosporin A[5,6].

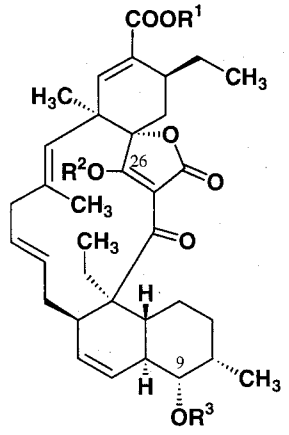
Structure Elucidation of Decatromicin B (2)

The molecular formula of decatromicin B (**2**) was established as $C_{45}H_{56}Cl_2N_2O_{10}$ by HRFAB-MS data [found *m/z* 855.3364 $(M+H)^+$, calcd. *m/z* 855.3390 for

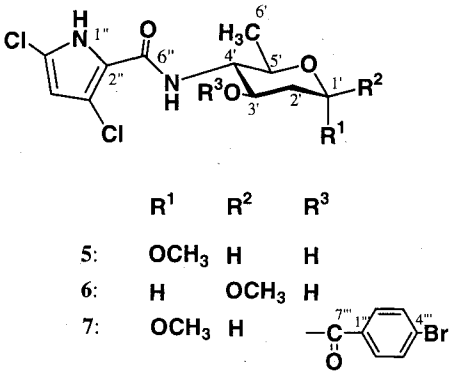
Table 1. ^{13}C and ^{1}H NMR assignments of decatromicins A (**1**) and B (**2**) in CD_3OD.

	1		**2**	
Position	^{13}C ppm (mult.)	^{1}H ppm (mult., J(Hz))	^{13}C ppm (mult.)	^{1}H ppm (mult., J(Hz))
1	169.00 (s)		169.99 (s)	
2	104.34 (s)		103.94 (s)	
3	206.02 (s)		205.29 (s)	
4	56.07 (s)		55.97 (s)	
5	41.67 (d)	1.84 (m)	41.78 (d)	1.82 (m)
6	23.93 (t)	1.38 (m)	24.00 (t)	1.33 (m)
		1.79 (m)		1.80 (m)
7	33.23 (t)	1.63 (m)	33.24 (t)	1.61 (m)
		1.63 (m)		1.61 (m)
8	35.47 (d)	2.40 (m)	35.47 (d)	2.38 (m)
9	87.38 (d)	3.38 (dd, 5.0, 10.8)	87.40 (d)	3.38 (dd, 5.2, 11.2)
10	39.73 (d)	2.16 (br dd, 10.0, 10.8)	39.78 (d)	2.15 (br dd, 10.0, 11.2)
11	125.58 (d)	5.67 (br d, 10.2)	125.43 (d)	5.63 (br d, 10.4)
12	132.50 (d)	5.71 (ddd, 2.2, 5.8, 10.2)	132.69 (d)	5.69 (ddd, 2.2, 5.8, 10.4)
13	44.16 (d)	2.88 (m)	44.00 (d)	2.89 (m)
14	38.01 (t)	2.02 (m)	38.01 (t)	1.99 (m)
		2.02 (m)		1.99 (m)
15	132.50 (d)	5.19 (m)	132.65 (d)	5.21 (m)
16	129.26 (d)	5.43 (ddd, 5.2, 9.8, 14.8)	129.10 (d)	5.42 (ddd, 4.8, 10.0, 16.0)
17	45.77 (t)	2.36 (dd, 5.2, 11.6)	45.62 (t)	2.36 (4.8, 12.0)
		2.50 (dd, 9.8, 11.6)		2.48 (10.0, 12.0)
18	140.35 (s)		140.16 (s)	
19	126.68 (d)	4.99 (br s)	126.86 (d)	4.99 (br s)
20	43.85 (s)		43.89 (s)	
21	143.72 (d)	7.07 (d, 1.8)	144.10 (d)	7.05 (d, 1.6)
22	132.90 (s)		132.83 (s)	
23	37.16 (d)	2.71 (m)	37.23 (d)	2.68 (m)
24	31.06 (t)	1.83 (d, 14.2)	30.98 (t)	1.82 (d, 14.2)
		2.49 (dd, 9.0, 14.2)		2.43 (dd, 9.0, 14.2)
25	87.01 (s)		86.86 (s)	
26	200.14 (s)		200.45 (s)	
27	24.16 (t)	1.88 (m)	24.13 (t)	1.84 (m)
		2.73 (m)		2.72 (m)
28	12.23 (q)	0.91 (t, 7.6)	12.28 (q)	0.91 (t, 7.6)
29	13.54 (q)	1.03 (d, 7.2)	13.51 (q)	1.02 (d, 7.2)
30	19.16 (q)	1.78 (d, 1.2)	19.12 (q)	1.79 (d, 1.0)
31	26.90 (q)	1.31 (s)	26.86 (q)	1.30 (s)
32	170.43 (s)		170.57 (s)	
33	27.36 (t)	1.54 (m)	27.33 (t)	1.59 (m)
		1.75 (m)		1.72 (m)
34	13.22 (q)	0.92 (t, 7.6)	13.26 (q)	0.91 (t, 7.9)
1'	103.00 (d)	4.52 (dd, 1.6, 10.0)	102.86 (d)	4.55 (dd, 1.8, 9.8)
2'	41.24 (t)	1.58 (m)	41.13 (t)	1.57 (m)
		2.27 (ddd, 1.6, 4.8, 12.6)		2.26 (ddd, 1.6, 5.0, 12.6)
3'	70.26 (d)	3.68 (ddd, 4.8, 10.0, 11.0)	70.30 (d)	3.72 (ddd, 5.0, 10.0, 12.0)
4'	59.43 (d)	3.54 (t, 10.0)	59.74 (d)	3.58 (t, 10.0)
5'	72.45 (d)	3.39 (m)	72.44 (d)	3.45 (qd, 6.6, 10.0)
6'	18.71 (q)	1.20 (d, 6.0)	18.79 (q)	1.23 (d, 6.6)
2"	126.86 (s)		122.56 (s)	
3"	119.91 (s)		119.58 (s)	
4"	108.44 (d)	6.03 (d, 4.0)	109.33 (d)	6.11 (s)
5"	112.66 (d)	6.79 (d, 4.0)	114.67 (s)	
6"	163.24 (s)		161.68 (s)	

Fig. 3. Methanolysis products of **2** and their derivative.



	R^1	R^2	R^3
3:	H	H	H
4:	CH_3	H	H
8:	CH_3	CH_3	H
9:	CH_3	CH_3	(*R*)-MTPA
10:	CH_3	CH_3	(*S*)-MTPA



	R^1	R^2	R^3
5:	OCH_3	H	H
6:	H	OCH_3	H
7:	OCH_3	H	

MTPA: 2-methoxy-2-phenyl-2-(trifluoromethyl)acetyl

$C_{45}H_{57}Cl_2N_2O_{10}$] and NMR spectra. The molecular formula of **2** indicated the presence of one more chlorine and one less proton atoms compared with that of **1**. The ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1** (Table 1). However, the corresponding signal to H-5″ (δ 6.79) was not observed in ^1H NMR spectrum of **2**. Therefore, the structure of **2** was determined as a 5″-chloro analogue of **1** (Fig. 1).

Conversion of Decatromicin B (2) into Decatromicin A (1)

Compared with **2**, **1** lacks a chlorine atom on the pyrrole ring. Dechlorination of **2** was carried out by heating **2** with tri-*n*-butylstannane (TBTH) in the presence of a catalytic amount of α,α'-azobisisobutyronitrile (AIBN) at 140°C for 24 hours to afford 5″-dechloro compound (**1**) ($\delta_{4''}$ 6.03 and $\delta_{5''}$ 6.79, 12.1%), 3″-dechloro compound (**11**) ($\delta_{4''}$ 6.18 and $\delta_{3''}$ 6.88, 29.1%), 3″,5″-didechloro compound (**12**) (δ 6.15, 6.82 and 6.89, 10.1%) along with an aglycone **3** (14.1%) and a starting **2** (23.3%). Details of the dechlorinated **11** and **12** will be reported in due course with another derivatives. The spectroscopic data and optical rotation of synthetic **1** were in full agreement with those of natural **1**.

Relative Configuration of Aglycone (3)

The planar structure of **2** differs from pyrrolosporin A by having an additional methyl group at C-18, which plays an important role in the determination of the stereochemistry of macrocyclic ring. Although the determination of relative structure of several other members of this family by an X-ray analysis were reported[2,6,7], to the best of our knowledge, there have not been done by NMR method. We considered that the formation of an intramolecular hydrogen bond between a carbonyl group and an enolic hydroxy group made it become impossible to determine the stereochemistry of the spiro center (*i.e.* C-25) by NOESY analysis. In order to prove this assumption, we decided to convert it to enol ether to confirm its relative configuration by NMR techniques.

To reduce the number of overlapped signals, degradation of **2** was carried out. Methanolysis of **2** with 2.7 M hydrogen chloride in methanol at room temperature gave aglycone **3**, methyl ester of aglycone **4**, α-glycoside **5** and β-glycoside **6** (Fig. 3). Treatment of **3** with diazotrimethylsilylmethane afforded methyl enol ether **8** (OCH_3 at δ 3.81 and CO_2CH_3 at δ 3.77) in good yield. The ^1H and ^{13}C NMR data of **3**, **4** and **8** are shown in Tables 2 and 3. Fortunately, 26-OCH_3

Table 2. ^{1}H NMR assignments of **3**, **4** and **8** in $CDCl_3$.

Proton	**3** ppm (mult., *J* (Hz))	**4** ppm (mult., *J* (Hz))	**8** ppm (mult., *J* (Hz))
5	1.79 (m)[a]	1.79 (m)[a]	1.87 (m)[a]
6ax	1.38 (m)	1.38 (m)	1.34 (m)
6eq	1.82 (m)[a]	1.82 (m)[a]	
7	1.67 (m)	1.66 (m)	1.61 (m)
8	2.21 (m)	2.20 (m)	2.17 (m)
9	3.53 (dd, 5.2, 10.3)	3.52 (dd, 5.2, 10.4)	3.48 (dd, 5.2, 10.4)
10	2.09 (br dd, 10.3, 11.0)	2.08 (ddd, 1.6, 11.0, 12.0)	2.01 (m)[a]
11	5.82 (br d, 10.0)	5.81 (br d, 10.0)	5.83 (br d, 10.4)
12	5.71 (ddd, 2.4, 5.6, 10.0)	5.71 (ddd, 2.4, 5.6, 10.0)	5.64 (ddd, 2.4, 5.6, 10.4)
13	2.88 (br t, 7.0)	2.88 (br t, 7.0)	3.26 (m)
14	2.01 (m)	2.01 (m)	2.05 (m)[a]
15	5.19 (ddd, 4.8, 9.6, 14.8)	5.19 (ddd, 4.8, 9.6, 14.8)	5.18 (m)
16	5.38 (dddd, 2.0, 4.2, 10.0, 14.8)	5.40 (dddd, 2.0, 4.2, 10.0, 14.8)	5.43 (m)
17a	2.34 (dd, 5.6, 12.0)	2.33 (br dd, 5.6, 12.0)	2.38 (br dd, 5.6, 12.0)
17b	2.47 (dd, 10.0, 12.0)	2.46 (dd, 10.0, 12.0)	2.47 (dd, 10.0, 12.0)
19	5.0 (br s)	5.0 (d, 1.4)	5.0 (br s)
21	7.22 (d, 1.6)	7.03 (d, 1.6)	6.96 (d, 2.0)
23	2.71 (m)	2.73 (m)	2.71 (m)
24ax	2.44 (dd, 8.4, 14.8)	2.43 (dd, 8.4, 14.8)	2.56 (dd, 10.4, 14.8)
24eq	1.83 (d, 14.8)	1.81 (d, 14.8)	1.73 (d, 14.8)
27a	1.89 (dq, 8.0, 15.6)	1.89 (dq, 8.0, 15.6)	1.91 (dq, 7.8, 15.6)
27b	2.77 (dq, 8.0, 15.6)	2.77 (dq, 8.0, 15.6)	2.48 (dq, 7.8, 15.6)
28	0.88 (t, 8.0)	0.89 (t, 8.0)	0.99 (t, 7.8)
29	1.02 (d, 7.0)	1.02 (d, 7.0)	0.98 (d, 7.0)
30	1.75 (d, 0.6)	1.75 (d, 0.6)	1.68 (d, 0.5)
31	1.33 (s)	1.31 (s)	1.30 (s)
33a	1.54 (m)	1.51 (m)	1.20 (m)
33b	1.78 (m)[a]	1.69 (m)[a]	1.82 (m)
34	0.92 (t, 7.6)	0.91 (t, 7.6)	0.99 (t, 7.8)
26-OCH_3			3.81 (s)
CO_2CH_3		3.79 (s)	3.77 (s)
OH	16.37	16.34	

[a] These signals overlap with other signals.

showed interactions to 30-H (δ 1.68), 6-H (δ 1.34), 33-H_a (δ 1.20) and 28-H (δ 0.99) in NOESY spectrum of **8**. This result encouraged us to elucidate the relative and absolute configuration of the aglycone by NMR techniques for the MTPA derivatives of **8**. The (*R*)-MTPA derivative **9** and (*S*)-MTPA derivative **10** were prepared by treatment of **8** with (+)- and (−)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetyl chlorides (MTPA-Cl), respectively, in CH_2Cl_2 in the presence of triethylamine and catalytic amount of 4-dimethylaminopyridine (DMAP). Table 4 summarizes ^{1}H and ^{13}C NMR assignments of **9** and **10**.

The relative structure of **10** was determined by ^{1}H, HMBC and NOESY NMR experiments.

Octalin ring (Fig. 4): In ^{1}H NMR spectrum of **10**, the large coupling constants ($J_{5,10}$=10.7 and $J_{9,10}$=11.2 Hz) confirmed the *trans*-diaxial relationship between 10-H (δ 2.30) and both 5-H (δ 1.98) and 9-H (δ 4.86). The equatorial 8-H (δ 2.43) was determined not only by NOESY correlation between 8-H and 9-H but by small *J* values ($J_{7ax,8}$=4.9 Hz, $J_{7eq,8}$=2.5 Hz and $J_{8,9}$=4.9 Hz) as well. In addition, the observed NOESY correlations between 10-H and both 29-H (δ 0.73) and 27-H_a (δ 1.85), and between 27-H_b (δ 2.48) and 13-H (δ 3.26) indicated that these protons were on the same face of the ring. Therefore, the cyclohexane ring in octalin moiety has a chair form with an axial methyl group at C-8 and an equatorial acyloxy group at C-9. The cyclohexene ring in octalin moiety takes a half-chair form with an axial ethyl

Table 3. ^{13}C NMR assignments of **3**, **4** and **8** in $CDCl_3$.

Carbon	3	4	8
1	166.45	166.48	168.61
2	102.79	102.83	109.07
3	206.05	206.06	199.07
4	54.70	54.70	56.00
5	39.74	39.73	40.11
6	22.91	22.91	21.55
7	32.16	32.16	31.89
8	34.55	34.58	34.46
9	76.36	76.33	76.30
10	39.58	39.60	38.78
11	123.84	123.83	124.34
12	131.48	131.49	132.55
13	42.95	42.95	40.36
14	36.68	36.68	37.14
15	131.60	131.56	130.06
16	127.55	127.61	132.00
17	44.61	44.64	44.60
18	139.11	138.99	142.01
19	125.14	125.32	124.60
20	42.76	42.58	43.49
21	145.21	142.73	142.62
22	130.28	130.96	132.43
23	35.55	35.78	35.58
24	30.03	30.11	30.79
25	85.40	85.51	85.77
26	200.33	200.35	189.04
27	23.11	23.11	23.54
28	11.80	11.80	13.10
29	11.95	11.94	11.72
30	18.83	18.79	17.10
31	26.39	26.49	28.25
32	171.99	167.67	167.92
33	26.15	26.25	28.13
34	13.00	13.01	13.47
26-OCH_3			63.68
CO_2CH_3		51.88	51.73

group at C-4 and a pseudo-equatorial proton at C-13.

Macrocyclic ring (Fig. 5): Since ^1H-^{13}C long-range correlation from C-26 (δ 189.22) to both 26-OCH_3 (δ 63.68) and 24-H_2 (δ 2.56 and 1.74) were observed in HMBC spectra of **10**, the position of the methoxy group was indicated to be at C-26. The geometry of the Δ^{15} and Δ^{18} double bonds were assigned as *E* on the basis of large *J* value between 15-H (δ 5.16) and 16-H (δ 5.44) ($J_{15,16}$=15.1 Hz) and NOESY correlations between 15-H and 19-H (δ 5.00) and between 16-H and 30-H, (δ 1.68) respectively. In agreement with this geometry, the stereochemistry of macrocyclic ring was established by NOESY spectra. The 26-OCH_3 showed significant NOESY correlations with 30-H and 33-H_a (δ 1.19). No NOESY correlation could be observed between 26-OCH_3 and 31-H (δ 1.31). On the other hand, NOESY correlations between 15-H and both 13-H and 19-H and between 19-H and 31-H were observed. It is evident that protons 13-H, 15-H, 17-H_b (δ 2.47), 19-H, 31-H (20-CH_3) and protons 16-H, 17-H_a, 30-H (18-CH_3) and 26-OCH_3 are on the opposite face of the ring.

Cyclohexene (Fig. 6): The ethyl group positioned pseudo-axial to reduce the allylic strain ($A^{1,2}$ strain)[8]. The cyclohexene ring was assigned as half-chair conformation on the basis of ^1H-^1H coupling constants between 23-H (δ 2.71) and both 24-H_{eq} (δ 1.74) and 24-H_{ax} (δ 2.56) ($J_{23,24eq}$=0 Hz, $J_{23,24ax}$=9.8 Hz). An observed NOESY correlation between 34-H (δ 0.99) and 24-H_{eq} indicated that both protons were the *syn* relationship. On the other hand, NOESY correlations between 31-H, 24-H_{ax} and 23-H showed that these protons were on the same face of the ring (*i.e.* 20-CH_3 positioned pseudo-axial).

Amino sugar (Fig. 3): The relative stereochemistry of **5** was determined by the analysis of the ^1H-^1H coupling constants and NOESY experiments. The anomeric configuration of the methyl glycoside **5** ($[\alpha]_D$ +85°) was found to be α, because the vicinal coupling constants of the broad doublet of 1′-H (δ 4.79) were <1.0Hz and 3.0 Hz. The coupling constants between 2′-H_{ax} (δ 1.75) and 3′-H (δ 3.98), 3′-H and 4′-H (δ 3.84), and 4′-H and 5′-H (δ 3.74) were 9.8 Hz, respectively. In NOESY spectrum, cross peaks were observed between 2′-H_{ax} and 4′-H, and 3′-H and 5′-H, respectively. These observations indicated that the conformation of sugar part is a C1 chair-form with equatorial substituents. The other methyl glycoside **6** ($[\alpha]_D$ −20°) was established to be β-anomer by the analysis of ^1H-NMR spectrum.

Absolute Configuration

Aglycone: The secondary hydroxy group at C-9 allowed the application of the modified Mosher's method[9] to confirm the absolute configuration at that center. By chemical shift differences ($\Delta\delta=\delta_{10}-\delta_9$) between a diastereomeric couple of 9-*O*-MTPA derivatives **9** and **10** illustrated in Fig. 7, the absolute configuration of C-9 was concluded to be *S*.

Amino sugar: α-Glycoside **5** was treated with *p*-bromobenzoyl chloride in pyridine in the presence of DMAP to give colorless prisms **7** that was recrystallized from ethyl ether. The absolute stereochemistry of **7** was determined by the X-ray crystallographic analysis using anomalous scattering of the bromine atom. Thus, the amino

Table 4. ^{13}C and ^{1}H NMR assignments of MTPA derivatives (**9**) and (**10**) in $CDCl_3$.

Position	**9**		**10**	
	^{13}C	^{1}H ppm (Mult., *J* (Hz))	^{13}C	^{1}H ppm (Mult., *J* (Hz))
1	168.56		168.58	
2	108.94		108.93	
3	198.58		198.55	
4	55.89		55.89	
5	40.43	1.965 (ddd, 2.5, 10.4, 10.7)	40.44	1.980 (ddd, 2.5, 10.4, 10.7)
6	21.19	*	21.23	*
7	31.60	ax 1.731 (m)[a]	31.56	ax 1.723 (m)[a]
		eq 1.592 (m)		eq 1.559 (m)
8	31.60	2.416 (dddq, 2.5, 4.9, 4.9, 7.0)[a,b]	31.17	2.403 (dddq, 2.5, 4.9, 4.9, 7.0)[a,b]
9	81.70	4.911 (dd, 4.9, 11.2)	81.99	4.859 (dd, 4.9, 11.2)
10	36.15	2.274 (br dd, 10.7, 11.2)	35.97	2.302 (br dd, 10.7, 11.2)
11	122.67	5.129 (br d, 10.0)	122.88	5.379 (br d, 9.8)
12	133.17	5.501 (ddd, 2.4, 5.9, 9.8)	133.39	5.595 (ddd, 2.4, 5.9, 9.8)
13	40.27	3.228 (m)	40.28	3.261 (m)
14	36.96	2.036 (m)	36.94	2.051 (m)
15	129.85	5.145 (br dt, 7.3, 15.1)	129.80	5.157 (br dt, 7.3, 15.1)
16	132.19	5.434 (br ddd, 6.0, 10.0, 15.1)	132.23	5.443 (br ddd, 6.0, 10.0, 15.1)
17	44.60	a 2.385 (br dd, 6.0, 11.3)	44.60	a 2.391 (br dd, 6.0, 11.3)
		b 2.462 (br dd, 10.0, 11.3)[a]		b 2.473 (br dd, 6.0, 11.3)[a]
18	142.02		142.02	
19	124.59	4.994 (br s)	124.60	5.002 (br s)
20	43.49		43.49	
21	142.59	6.963 (d, 2.0)	142.58	6.967 (d, 2.0)
22	132.43		132.43	
23	35.57	2.707 (m)	35.57	2.710 (m)
24	30.79	ax 2.550 (br dd, 9.8, 14.2)	30.79	ax 2.555 (br dd, 9.8, 14.2)
		eq 1.732 (br d, 14.2)		eq 1.739 (br d, 14.2)
25	85.83		85.85	
26	189.19		189.22	
27	23.41	a 1.823 (dq, 7.8, 16.3)	23.43	a 1.852 (dq, 7.8, 16.3)
		b 2.462 (dq, 7.8, 16.3)		b 2.479 (dq, 7.8, 16.3)
28	13.09	0.971 (t, 7.8)	13.08	0.974 (t, 7.8)
29	12.40	0.936 (d, 7.0)	11.99	0.727 (d, 6.8)
30	17.10	1.675 (s)	17.11	1.681 (s)
31	28.25	1.302 (s)	28.25	1.308 (s)
32	167.89		167.89	
33	28.13	a 1.190 (m)	28.13	a 1.192 (m)
		b 1.825 (m)[a]		b 1.829 (m)[a]
34	13.45	0.989 (t, 7.3)	13.45	0.991 (t, 7.3)
26-OCH_3	63.67	3.801 (s)	63.68	3.801 (s)
CO_2CH_3	51.74	3.767 (s)	51.74	3.769 (s)

* Complex signal
[a] These signals overlap with other signals
[b] Confirmed by decoupling difference spectroscopy

sugar moiety in **5** was found to be the methyl 4-amino-2,4,6-trideoxy-β-D-*arabino*-hexopyranoside, designated as pyrrosamine[6].

From all data described above, the absolute structures of decatromicins A (**1**) and B (**2**) were completely determined as shown in Fig. 1.

BE-45722s, one of which has the same planar structure as **2**, were very recently claimed in patent by Banyu group[10].

Fig. 4. NOESY experiments of octalin part of **10**.

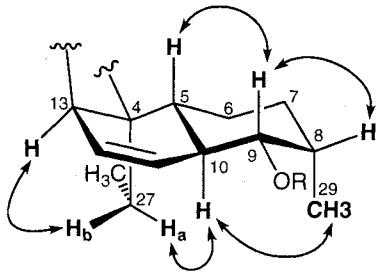


Fig. 5. NOESY and HMBC experiments of macrocyclic part of **10**.

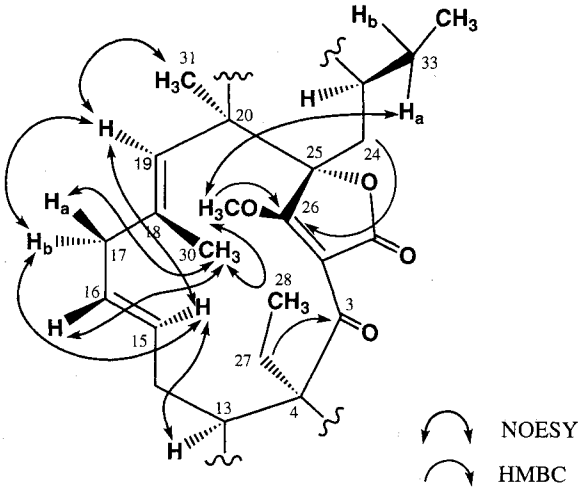


Fig. 6. NOESY experiments of cyclohexene part of **10**.

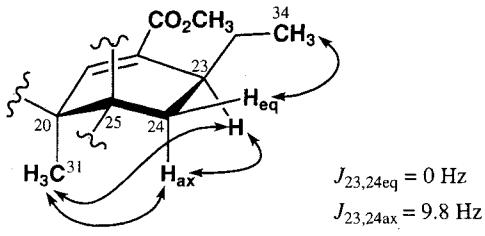
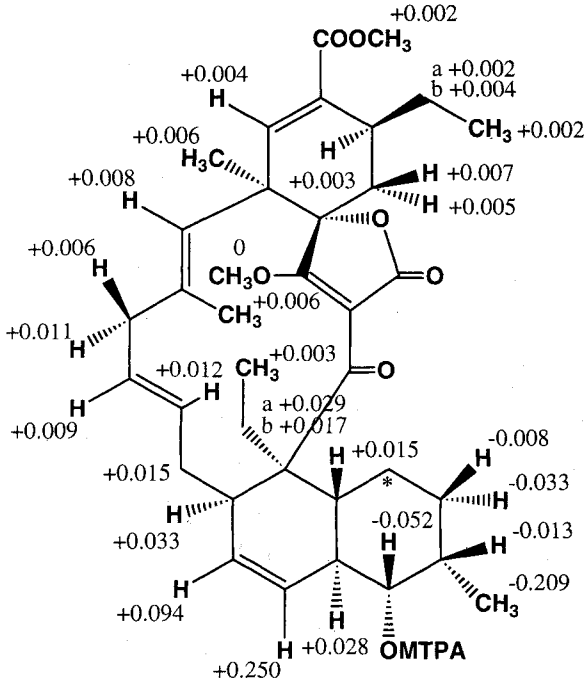


Fig. 7. $\Delta\delta$ Values between **9** and **10**.



Experimental

General

NMR spectra were obtained on a JEOL JNM-A500 spectrometer at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. Chemical shifts are given in ppm using TMS as an internal standard. UV absorption spectra were measured with a Hitachi U-3210 spectrophotometer. FAB-MS and HRFAB-MS were measured with a JEOL JMS-SX 102 spectrometer. Optical rotations were taken by a Perkin-Elmer 241 polarimeter.

Methanolysis of **2**

A suspension of **2** (28.4 mg) in 2.7 M HCl-MeOH (1 ml) was stirred at room temperature for 9 days. The reaction mixture was dried under reduced pressure. The oily residue was chromatographed on silica gel (Wakogel C-200, hexane - EtOAc, 25 : 1, 10 : 1, 4 : 1, 1 : 1 stepwise) to give **3** (4.9 mg), **4** (6.6 mg) and an anomeric mixture (**5** and **6**). The glycosidic mixture was purified by reverse phase HPLC under the following condition: column; PEGASIL ODS (Senshu Scientific Co., Ltd., 20.0×250 mm), mobile phase; CH_3CN-H_2O (3 : 7), flow rate; 10.0 ml/minute, detection; UV at 220 nm. Fractions containing **5** were collected and concentrated under reduced pressure to give a

colorless oily methyl α-glycoside **5** (5.2 mg). β-Glycoside **6** (1.0 mg) was also obtained as an oil.

3: $[\alpha]_D^{25}$ −41.7° (*c* 0.30, MeOH). HRFAB-MS *m/z* 565.3188 $(M+H)^+$ (calcd *m/z* 565.3165 for $C_{34}H_{45}O_7$).

4: $[\alpha]_D^{23}$ −43.8° (*c* 0.60, MeOH). HRFAB-MS *m/z* 579.3325 $(M+H)^+$ (calcd *m/z* 579.3322 for $C_{35}H_{47}O_7$).

5: $[\alpha]_D^{22}$ +85.0° (*c* 0.50, MeOH). HRFAB-MS *m/z* 323.0556 (calcd *m/z* 323.0565 for $C_{12}H_{17}Cl_2N_2O_4$). ^{1}H NMR ($CDCl_3$) δ 4.79 (1H, br d, *J*=3.0 Hz, 1′-H), 3.34 (3H, s, 1′-OCH_3), 1.75 (1H, ddd, *J*=3.0, 9.8, 13.2 Hz, 2′-Hax), 2.26 (1H, dd, *J*=4.0, 13.2 Hz, 2′-Heq), 3.98 (1H, dt, *J*=4.0, 9.8 Hz, 3′-H), 3.84 (1H, br q, *J*=9.8 Hz, 4′-H), 6.52 (1H, br d, *J*=8.8 Hz, 4′-NH), 3.74 (1H, qd, *J*=6.2, 9.8 Hz, 5′-H), 1.29 (3H, d, *J*=6.2 Hz, 6′-H) and 6.04 (1H, s, 4″-H). ^{13}C NMR ($CDCl_3$) δ 98.56 (C-1′), 54.90 (1′-OCH_3), 38.45 (C-2′), 68.63 (C-3′), 58.96 (C-4′), 66.57 (C-5′), 18.25 (C-6′), 120.23 (C-2″), 119.30 (C-3″), 108.90 (C-4″), 113.22 (C-5″) and 160.57 (C-6″).

6: $[\alpha]_D^{24}$ −20.0° (*c* 0.20, MeOH). HRFAB-MS *m/z* 323.0591 (calcd *m/z* 323.0565 for $C_{12}H_{17}Cl_2N_2O_4$). ^{1}H NMR ($CDCl_3$) δ 4.40 (1H, br d, *J*=9.2 Hz, 1′-H), 3.48 (3H, s, 1′-OCH_3), 1.65 (1H, br dd, *J*=10.2, 11.8 Hz, 2′-Hax), 2.33 (1H, br d, *J*=11.8 Hz, 2′-Heq), 3.79 (1H, m, 3′-H), 3.79 (1H, m, 4′-H), 6.49 (1H, br d, *J*=7.8 Hz, 4′-NH), 3.79 (1H, m, 5′-H), 1.34 (3H, d, *J*=6.2 Hz, 6′-H) and 6.07 (1H, s, 4″-H). ^{13}C NMR ($CDCl_3$) δ 100.68 (C-1′), 56.44 (1′-OCH_3), 39.63 (C-2′), 71.38 (C-3′), 58.94 (C-4′), 70.76 (C-5′), 18.26 (C-6′), 120.21 (C-2″), 119.32 (C-3″), 109.11 (C-4″), 113.30 (C-5″) and 160.84 (C-6″).

p-Bromobenzoylation of **5**

α-Glycoside **5** (5.2 mg) in pyridine (0.2 ml) was treated with *p*-bromobenzoyl chloride (3.5 mg) and DMAP (0.05 mg) for 18 hours at room temperature. To the reaction mixture, 1 M HCl and EtOAc were added. The EtOAc solution was washed with aqueous sodium hydrogen carbonate and water. After evaporation the solvent, the residue was purified by a silica gel TLC (hexane : EtOAc = 3 : 1) to give **7** (3.8 mg) as colorless prisms. $[\alpha]_D^{24}$ −3.80° (*c* 0.21, MeOH). HRFAB-MS *m/z* 504.9940 (calcd *m/z* 504.9933 for $C_{19}H_{20}BrCl_2N_2O_5$). ^{1}H NMR ($CDCl_3$) δ 4.88 (1H, br d, *J*=3.6 Hz, 1′-H), 3.39 (3H, s, 1′-OCH_3), 1.96 (1H, ddd, *J*=3.6, 11.6, 12.4 Hz, 2′-Hax), 2.36 (1H, ddd, *J*=1.4, 5.2, 12.4 Hz, 2′-Heq), 5.46 (1H, dt, *J*=5.2, 10.2 Hz, 3′-H), 4.21 (1H, q, *J*=10.2 Hz, 4′-H), 6.52 (1H, br d, *J*=9.8 Hz, 4′-NH), 3.86 (1H, qd, *J*=6.2, 10.2 Hz, 5′-H), 1.32 (3H, d, *J*=6.2 Hz, 6′-H), 6.04 (1H, d, *J*=3.4 Hz, 4″-H), 7.80 (2-H, d, *J*=8.6 Hz, 2‴, 6‴-H) and 7.49 (2-H, d, *J*=8.6 Hz, 3‴, 5‴-H). ^{13}C NMR ($CDCl_3$) δ 98.07 (C-1′), 54.91 (1′-OCH_3), 35.74 (C-2′), 70.10 (C-3′), 55.39 (C-4′), 67.58 (C-5′), 18.19 (C-6′), 120.37 (C-2″), 118.67 (C-3″), 109.08 (C-4″), 113.14 (C-5″), 159.13 (C-6″), 128.67 (C-1‴), 131.24 (C-2‴, 6‴), 131.67 (C-3‴, 5‴), 128.27 (C-4‴) and 165.51 (C-7‴).

X-Ray Crystallography

Crystals of **7** were obtained from an ethyl ether solution. A colorless prism crystal having approximate dimensions of 0.03×0.20×0.25 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation and a rotating anode generator. Crystal data are as follows. $C_{19}H_{19}N_2O_5BrCl_2 \cdot 1/4H_2O$, formula weight: 510.68, crystal system: orthorhombic, space group: $P2_12_12_1$ a=17.527(4) Å, b=17.539(6) Å, c=15.950(2) Å, V=49303(1) Å^3, Z=8, D_{calc}=1.384 g/cm^3, μ(CuKα)=45.62 cm^{-1}. Of the 6410 reflections which were collected, 3778 were unique (R_{int}=0.061). No decay correction was applied. The structure was solved by direct methods[11] and expanded using Fourier techniques[12]. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 2820 observed reflections ($I>1.5\sigma(I)$) and 532 variable parameters and converged with unweighted and weighted agreement factors of R=0.074 and R_w=0.105. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.86 and −0.17e^-/Å^3, respectively.

Comparing $\frac{|F_0(hkl)|}{|F_0(\bar{h}\bar{k}\bar{l})|}$ and $\frac{|F_c(hkl)|}{|F_c(\bar{h}\bar{k}\bar{l})|}$

for 70 Bijvoet mates for which the difference

$$\frac{\big||F_c(hkl)|-|F_c(\bar{h}\bar{k}\bar{l})|\big|}{\sqrt{\sigma^2(F_0(hkl))+\sigma^2(F_0(\bar{h}\bar{k}\bar{l}))}}$$

are longer than 1.0, 68 pairs showed consistently the absolute configuration in Fig. 3. All calculations were performed using the teXsan[13] crystallographic software package of Molecular Structure Corporation.

26-*O*-Methyl Aglycone Methyl Ester (**8**)

To a solution of **3** (28 mg, 0.05 mmol) in MeOH (0.2 ml) and $CHCl_3$ (0.8 ml) was added diazotrimethylsilylmethane (0.2 ml of a 10% hexane solution) under cooling. After stirring at the same temperature for 2 hours, the solvent was removed. The residue was chromatographed on silica gel with $CHCl_3$-MeOH (99 : 1) to give **8** (27 mg, 92%). $[\alpha]_D^{23}$ −34.0° (*c* 0.40, MeOH). HRFAB-MS *m/z* 593.3477 $(M+H)^+$ (calcd *m/z* 593.3478 for $C_{36}H_{49}O_7$).

9-*O*-(*R*)-MTPA Derivative (**9**)

(*S*)-MTPA chloride (5.7 μl, 0.03 mmol) was added to a solution of **8** (9 mg, 0.015 mmol) in CH_2Cl_2 (1 ml) in the presence of triethylamine (6.3 μl, 0.045 mmol) and catalytic amount of DMAP at room temperature. The reaction mixture was stirred for 4 hours. After evaporation, the residue was purified by preparative TLC with $CHCl_3$-MeOH (99 : 1) to give **9** (9.3 mg, 75.2%). FAB-MS *m/z* 809 $(M+H)^+$.

9-*O*-(*S*)-MTPA Derivative (**10**)

The (*S*)-MTPA derivative **10** (14.3 mg, 87.4%) was obtained from **8** (12 mg, 0.02 mmol) and (*R*)-MTPA chloride (7.5 μl, 0.06 mmol) by a similar procedure for **9**. FAB-MS *m/z* 809 $(M+H)^+$.

References

1) MOMOSE, I.; H. IINUMA, N. KINOSHITA, Y. MOMOSE, S. KUNIMOTO, M. HAMADA & T. TAKEUCHI: Decatromicins A and B, new antibiotics produced by *Actinomadura* sp. MK73-NF4. I. Taxonomy, isolation, physico-chemical properties and biological activities. J. Antibiotics 52: 781~786, 1999
2) MALLAMS, A. K.; M. S. PUAR & R. R. ROSSMAN: Kijanimicin. Part 3. Structure and absolute stereochemistry of kijanimicin. J. Chem. Soc. Perkin Trans. I. 1497~1534, 1983
3) HORAN, A. C.; M. C. SHEARER, V. HEGDE, M. L. BEYAZOA, B. C. BRODSKY, A. KING, R. BERRIE, K. CARDACI & M. NIMECK: A family of novel macrocyclic lactones, the saccarocarcins produced by *Saccarothrix aerocolonigenes* subsp. *antibiotica*. I. Taxonomy, fermentation, isolation and biological properties. J. Antibiotics 50: 119~125, 1997
4) HEGDE, V. R.; M. G. PATEL, P. R. DAS, B. PRAMANIK & M. S. PUAR: A family of novel macrocyclic lactones, the saccarocarcins produced by *Saccarothrix aerocolonigenes* subsp. *antibiotica*. II. Physico-chemical properties and structure determination. J. Antibiotics 50: 126~134, 1997
5) LAM, K. S.; G. A. HESLER, D. R. GUSTASON, R. L. BERRY, K. TOMITA, J. L. MACBETH, J. ROSS, D. MILLER & S. FORENZA: Pyrrolosporin A, a new antitumor antibiotic from *Micromonospora* sp. C39217-R109-7. I. Taxonomy of producing organism, fermentation and biological activity. J. Antibiotics 49: 860~864, 1996
6) SCHROEDER, D. R.; K. L. COLSON, S. E. KLOHR, M. S. LEE & J. A. MATSON: Pyrrolosporin A, a new antitumor antibiotic from *Micromonospora* sp. C39217-R109-7. II. Isolation, physico-chemical properties, spectroscopic study and X-ray analysis. J. Antibiotics 49: 865~872, 1996
7) MATSUMOTO, M.; Y. KAWAMURA, Y. YOSHIMURA, Y. TERUI, H. NAKAI, T. YOSHIDA & J. SHOJI: Isolation, characterization and structures of PA-46101A and B. J. Antibiotics 43: 739~747, 1990
8) JOHNSON, F. & S. K. MALHOTRA: Steric interference in allylic and pseudo-allylic systems. I. Two stereochemical theorems. J. Am. Chem. Soc. 87: 5492~5493, 1965
9) OHTANI, I.; T. KUSUMI, Y. KASHMAN & H. KAKISAWA: High field FTNMR application of Mosher's method. The absolute configuration of marine terpenoids. J. Am. Chem. Soc. 113: 4092~4096, 1991
10) TORIGOE, K.; S. NAKAJIMA, H. SUZUKI, M. NAGASHIMA, K. KOJIRI & H. SUDA (Banyu Pharmaceutical Co. Ltd.): Antibacterial substances BE-45722s. Jpn Kokai 9-227587, September 2, 1997
11) ALTOMARE, A.; M. C. BURLA, M. CAMALLI, M. CASCARANO, C. GIACOVAZZO, A. GUAGLIARDI & G. POLIDORI: SIR92-a program for automatic solution of crystal structure by direct methods. J. Appl. Cryst. 27: 435, 1994
12) BEURSKENS, P. T.; G. ADMIRAAL, G. BEURSKENS, W. P. BOSMAN, R. DE GELDER, R. ISRAEL & J. M. M. SMITH: The DIRDIF-94 program system. Technical report of the Crystallography Laboratory, University of Nijmegen, the Netherlands: 1994
13) Crystal structure analysis package, molecular structure corporation, 1985 & 1992