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IB-96212, a Novel Cytotoxic Macrolide Produced by a Marine *Micromonospora*

II. Physico-chemical Properties and Structure Determination

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IB-96212, is a new member of spiroketal containing macrolide class of fermentation-derived natural products isolated from mycelial extracts of *Micromonospora* sp. The structure consists of a new aglycone which possesses a 26-membered macrolide ring system and of one deoxy sugar identified as L-rhodinose, this structure represents the first reported spiroketal macrolide natural product related to other macrolides, such as oligomycins, dunaimycins, citovaricin, rutamycin and ossamycin.

IB-96212 is a fermentation-derived natural product with cytotoxic activity isolated from *Micromonospora* sp. The taxonomy, fermentation, isolation and biological activities have been described in the preceding paper[1]. Spectroscopic studies showed IB-96212 to have a spiroketal 26-membered macrocyclic lactone structure, related to oligomycins[2,3] and homooligomycins[4,5].

In this paper the physico-chemical properties and structure elucidation of IB-96212 are described.

Structural Elucidation of IB-96212

IB-96212 (**1**) was isolated as a pale white crystalline powder, mp 165~166°C. The physico-chemical properties of **1** are summarized in Table 1. The molecular formula of IB-96212 (**1**) was established as $C_{54}H_{94}O_{16}$ on the basis of HRFAB-MS data [*m/z* 1021.6443 $(M+Na)^+$, calcd. 1021.6439]. The IR spectrum indicated the existence of hydroxyl (3445 cm^{-1}) and carbonyl (1702 cm^{-1}) groups. The presence of conjugated diene system was also indicated by the IR (1641 cm^{-1}) and UV (λ_{max} 225 nm) spectra.

The ^1H and ^{13}C NMR spectral data of **1** are shown in Table 2 and Table 3 respectively. The ^{13}C NMR spectrum demostrated 54 signals which were assigned to eleven methyls, eleven methylenes, twenty-nine methines and three quaternary carbons by DEPT and HMQC experiments.

Detailed analysis of the ^1H-^1H COSY experiment proved the partial structures shown in Fig. 2, most of the protons

Table 1. Physico-chemical properties of IB-96212.

Appearance	Pale white crystalline powder
Molecular formula	$C_{54}H_{94}O_{16}$
HRFAB-MS $(M+Na)^+$	1021.6443 (Calcd; 1021.6439)
MP (°C)	165-166
$[\alpha]_D^{25}$	- 42.27° (c 0.22, $CHCl_3$)
UV λ_{max} nm	225
IR υ_{max}(KBr) cm^{-1}	3445, 1702, 1641
TLC[a] Rf value[b]	0.4
HPLC (Rt, minutes)[c]	3.6

a Silica gel 60 F_{254}, Merck

b Solvent : $CHCl_3$-MeOH (9:1)

c Resolve C18 radial pack cartridge (10μ); mobile phase: MeOH-H_2O (92:8); flow rate: 2 ml/min.; detection: 225 nm

Table 2. ^{1}H NMR assignments of IB-96212 (**1**) and aglycone (**2**) in acetone-d_6.

	1	**2**		**1**	**2**
position	δ_H [int mult, J (Hz)]		position	δ_H [int mult, J (Hz)]	
1			33	3.52 (1H, m)	3.55 (1H, m)
2	5.80 (1H, d, 15.5)	5.80 (1H, d, 15.5)	34	1.35 (1H, m)	1.37 (1H, m)
				1.68 (1H, m)	1.70 (1H, m)
3	6.74 (1H, dd, 10.5, 15.5)	6.75 (1H, dd, 10.0, 15.5)	35	0.94 (3H, t, 7.5)	0.95 (3H, t, 7.5)
4	2.37 (1H, dt, 6.5, 10.0)	2.45 (1H, dt, 6.5, 10.0)	36	1.13 (3H, d, 6.5)	1.14 (3H, d, 6.5)
5	3.62 (1H, d, 9.0)	3.65 (1H)	37	0.77 (3H, d, 7.0)	0.85 (3H, d, 7.0)
6	1.73 (1H, m)	1.75 (1H, m)	38	1.40 (1H, m)	1.45 (1H, m)
				1.64 (1H, m)	1.59 (1H, m)
7	4.02 (1H, d, 9.0)	3.94 (1H, d, 8.0)	39	1.42 (1H, m)	1.35 (1H, m)
				1.54 (1H, m)	1.55 (1H, m)
8	3.62 (1H, d, 9.0)	3.62 (1H)	40	0.91 (3H, t, 7.0)	0.88 (3H, t, 7.0)
9	3.54 (1H)	3.63 (1H)	41	0.96 (3H, d, 6.5)	0.98 (3H, d, 6.5)
10	4.07 (1H, dd, 4.5, 8.5)	4.14 (1H, d, 6.5)	42	1.25 (1H, m)	1.25 (1H, m)
				1.42 (1H, m)	1.45 (1H, m)
11	3.46 (1H)	3.50 (1H)	43	3.68 (1H, m)	3.80 (1H, m)
12			44	1.08 (3H, d, 6.5)	1.08 (3H)
13	3.78 (1H, dd, 1.5, 6.5)	3.78 (1H, broad s)	45	0.75 (3H, d, 7.0)	0.78 (3H, d, 7.0)
14	1.82 (1H, m)	1.85 (1H, m)	46	0.92 (3H, d, 7.0)	0.93 (3H, d, 7.0)
15	1.98 (1H, m)	2.10 (1H, m)	47	0.78 (3H, d, 6.0)	0.80 (3H, d, 6.0)
	2.19 (1H, m)	2.22 (1H, m)			
16	5.42 (1H, ddd, 3.5, 10.5, 14.5)	5.45 (1H, ddd, 3.5, 10.5, 14.5)	48	0.82 (3H, d, 7.0)	0.83 (3H, d, 7.0)
17	6.03 (1H, dd, 10.5, 14.5)	6.01 (1H, dd, 10.0, 14.5)	5-OH	4.21 (1H, s)	
18	6.01 (1H, dd, 10.5, 14.5)	6.08 (1H, dd, 10.0, 14.5)	7-OH	4.90 (1H, s)	
19	5.16 (1H, dd, 10.0, 14.5)	5.18 (1H, dd, 10.5, 14.5)	9-OH	3.50 (1H, s)	
20	2.26 (1H, dt, 10.0)	2.35 (1H, dt, 10.0)	10-OH	4.50 (1H, d, 4.5)	
21	1.48 (1H, m)	1.46 (1H, m)	11-OH	3.53 (1H, s)	
	1.64 (1H, m)	1.65 (1H, m)			
22	0.92 (1H, m)	1.02 (1H, m)	12-OH	3.75 (1H, s)	
	1.58 (1H, m)	1.60 (1H, m)			
23	3.92 (1H, d, 10.5)	3.96 (1H, d, 10.5)	13-OH	4.43 (1H, d, 6.5)	4.24 (1H, broad s)
24	1.95 (1H, m)	1.93 (1H, m)	33-OH	3.38 (1H, d, 6.0)	3.40 (1H, d, 6.4)
25	4.81 (1H, dd, 5.0, 11.5)	4.90 (1H, dd, 5.5, 11.5)	43-OH	3.39 (1H, d, 6.0)	3.43 (1H, d, 6.0)
26	1.75 (1H, m)	1.75 (1H, m)	1′	4.54 (1H, dd, 1.5, 7.7)	
27			2′	1.43 (1H, m)	
				2.09 (1H, dt, 2.0, 8.5)	
28	1.40 (1H, m)	1.40 (1H, m)	3′	1.46 (1H, m)	
	1.75 (1H, m)	1.77 (1H, m)		1.93 (1H, m)	
29	1.49 (1H, m)	1.50 (1H, m)	4′	3.15 (1H, sep, 5.0)	
	1.65 (1H, m)	1.65 (1H, m)			
30	1.49 (1H, m)	1.52 (1H, m)	5′	3.31 (1H, dq, 2.5, 6.0)	
31	3.82 (1H, d, 10.5)	3.84 (1H, d, 10.0)	6′	1.24 (3H, d, 6.0)	
32	1.66 (1H, m)	1.68 (1H, m)	4′-OH	4.04 (1H, d, 5.0)	

were assigned. The remaining protons and carbons were assigned by TOCSY and HMBC experiments.

The HMBC experiment of **1** showed the long range couplings of 2-H, 3-H and 25-H to C-1, of 5-H to C-7, of 5-OH to C-5, C-4 and C-6, of 11-H to C-9, C-12, C-13 and C-38, of 40-CH_3 to C-38, of 12-OH to C-12, C-11, C-13 and C-38, of 13-H to C-15 and 41-CH_3. Furthermore, the HMBC experiment also showed the long range couplings of 20-H to C-22, of 23-H to C-21, of 26-H, 28-H, 29-H and 46-CH_3 to C-27, of 34-H, 31-H, 35-CH_3 and 48-CH_3 to C-33. These correlations confirmed the linkages of the partial structures proved by ^{1}H-^{1}H COSY. And the presence of 26-membered macrocyclic lactone moety in **1** was established as Fig. 2. These results were also implied by FAB-MS fragmentation ion at *m/z* 885 corresponding to the aglycone fragment peak originated from the loss of sugar unit from

Table 3. ^{13}C NMR assignments of IB-96212 (**1**) and aglycone (**2**) in acetone-d_6.

	1	2		1	2
position	δ_C		position	δ_C	
1	165.2	165.3	28	33.1	32.8
2	122.7	122.5	29	28.8	28.8
3	150.7	150.7	30	31.7	31.7
4	42.5	42.0	31	74.1	74.2
5	80.8	80.6	32	39.9	39.9
6	36.4	36.5	33	73.6	73.6
7	77.5	78.6	34	28.8	28.8
8	83.5	74.0	35	9.7	9.7
9	71.6	73.0	36	18.4	18.2
10	69.1	71.0	37	4.5	4.9
11	70.9	72.1	38	38.0	38.4
12	76.7	77.5	39	17.2	17.2
13	68.0	70.6	40	15.2	15.2
14	34.0	34.3	41	15.0	15.1
15	39.2	39.2	42	46.9	46.7
16	131.6	131.5	43	65.3	65.4
17	133.1	133.2	44	24.8	24.8
18	132.1	132.3	45	6.7	6.5
19	137.5	137.5	46	12.4	12.3
20	41.8	41.2	47	18.2	18.1
21	33.1	33.1	48	9.8	9.8
22	32.2	31.8	1′	104.4	
23	68.9	68.7	2′	31.0	
24	36.7	37.0	3′	31.9	
25	76.9	76.6	4′	71.4	
26	38.7	38.8	5′	77.6	
27	99.1	99.1	6′	18.7	

Fig. 1. Structures of IB-96212 (**1**) and aglycone (**2**).

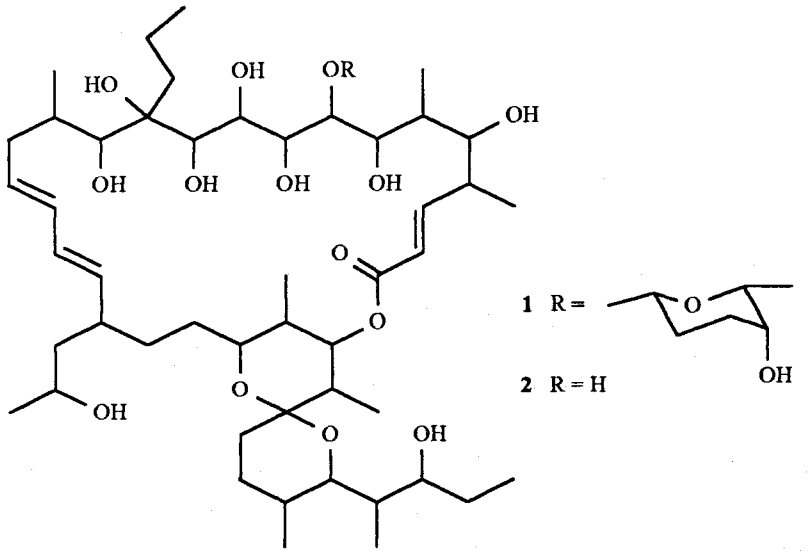
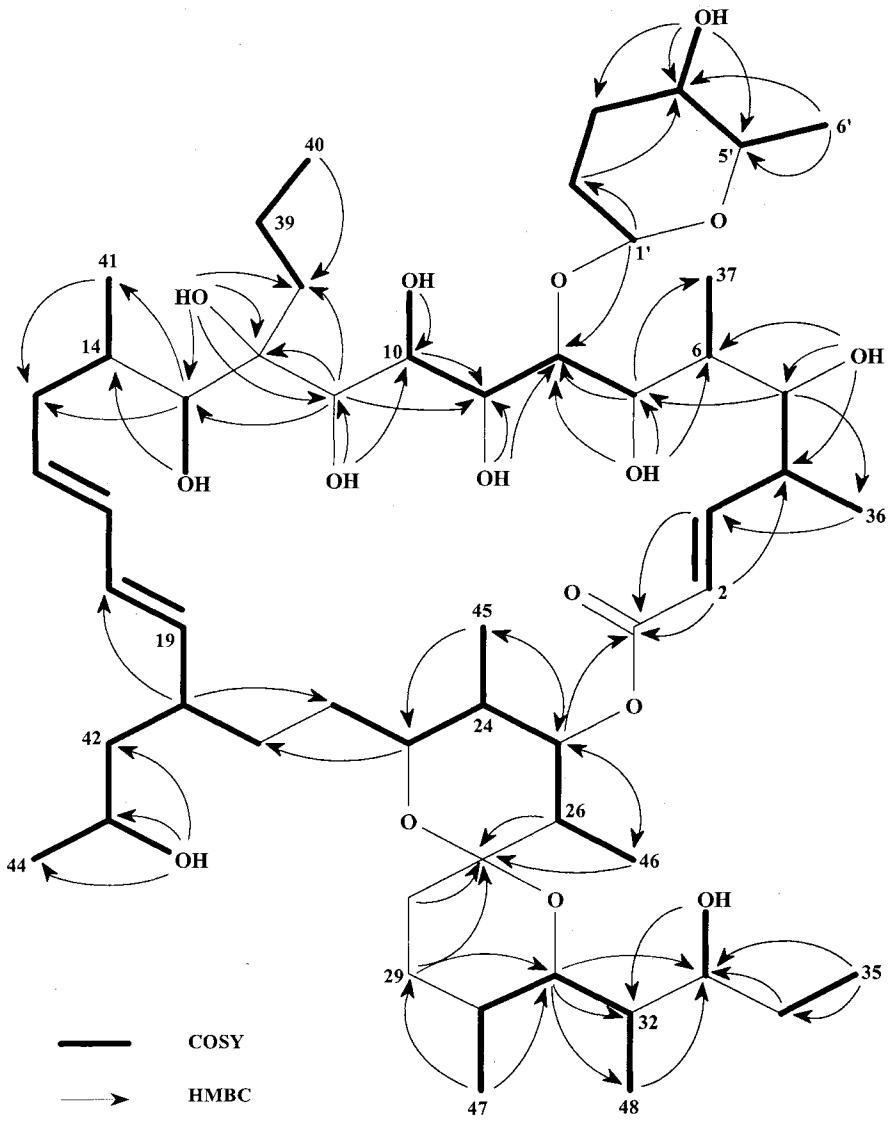


Fig. 2. Observed correlations in the ^{1}H-^{1}H COSY and HMBC experiments of IB-96212 (**1**).



the molecule.

The geometries of C-2, C-16 and C-18 were proved to be all *E* by the coupling constants of $J_{2,3}$=15.5 Hz, $J_{16,17}$=14.5 Hz and $J_{18,19}$=14.5 Hz, respectively.

The presence of the sugar was evident by the signal at 4.5 ppm in the ^{1}H NMR and by the signal at 104 ppm in the ^{13}C NMR spectrum corresponding to the anomeric proton and carbon respectively. The sugar sequence of a trideoxyhexose was also established using ^{1}H-^{1}H COSY and ^{13}C-^{1}H long range couplings. The sugar moiety was determined to be L-rhodinose, as a result of the ^{1}H and ^{13}C NMR chemical shifts and by analyzing the coupling constant between 5′-H and 4′-H, this constant is very small (J=2.5 Hz) which supports the equatorial (4′-H) - axial (5′-H) configuration[6,7]. The coupling constant values (J=7.7 and 1.5 Hz) for the anomeric proton $J_{1',2'ax}$ and $J_{1',2'eq}$ and the chemical shift of the anomeric carbon at δ 104.4 suggested that the sugar is attached to the aglycone by a β-glycoside bond[6,7]. On basis of NMR data, the sugar was determined to be β-L-rhodinose, the chemical shifts and coupling constants were compared with the reported values of L-rhodinose containing antibiotics, cirramycin F-1[6] and amicenomycins A and B[7]. The long range coupling of 1′-H (anomeric proton) to C-8 (δ 83.5) in the HMBC experiment showed the linkage of this sugar to the macrocyclic lactone ring at C-8.

The hydrolysis of **1** using aqueous 15% H_2SO_4 at reflux for 3 hours afforded the aglycone **2** which was purified by column chromatography. The FAB-MS spectrum showed *m/z* 885 $(M+H)^+$, this molecular weight of **2** suggested a loss of sugar compared to **1** and the structure of the aglycone **2** was confirmed by various NMR experiments including HMQC and HMBC (the ^1H and ^{13}C NMR data are shown in Table 2 and Table 3 respectively).

Experimental

General Procedures

Optical rotation was measured with an Optical Activity AA-10 polarimeter. Melting point was determined with a Mettler FP 81 mp apparatus and was uncorrected. IR and UV spectra were recorded on a Perkin Elmer 881 spectrophotometer and a Perkin Elmer Lambda 15 double beam spectrometer, respectively. NMR spectra were acquired on a Varian Unity 500 NMR spectrometer (500 MHz for ^1H, 125 MHz for ^{13}C) and Bruker DRX spectrometer (400 MHz for ^1H, 100 MHz for ^{13}C). Chemical shifts are reported in ppm referenced to the $(CH_3)_2CO$ peak at 2.05 ppm for ^1H and 29.8 and 206.2 ppm for ^{13}C. FAB-MS and HRFAB-MS were measured with a VG AUTOSPEG spectrometer.

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References

1) FERNÁMDEZ-CHIMENO, R. I.; L. CAÑEDO, F. ESPLIEGO, D. GRÁVALOS, F. DE LA CALLE, J. L. FERNÁNDEZ-PUENTES & F. ROMERO: IB-96212, a novel cytotoxic macrolide produced by a marine *Micromonospora*. I. Taxonomy, fermentation, isolation and biological Activities. J. Antibiotics 53: 474~478, 2000
2) CARTER, G. T.: Structure determination of oligomycins A and C. J. Org. Chem. 51: 4264~4271, 1986
3) LAATSCH, H.; M. KELLNER, G. WOLF, Y.-S. LEE, F. HANSSKE, S. KONETSCHNYRAPP, U. PESSARA, W. SCHEUER & H. STOCKINGER: Oligomycin F, a new immunosuppressive homologue of oligomycin A. J. Antibiotics 46: 1334~1341, 1993
4) YAMAZAKI, M.; T. YAMASHITA, T. HARADA, T. NISHIKIORI, S. SAITO, N. SHIMADA & A. FUJII: 44-Homooligomycins A and B, new antitumor antibiotics from *Streptomyces bottropensis*: Producing organism, fermentation, isolation, structure elucidation and biological properties. J. Antibiotics 45: 171~179, 1992
5) KIM, H. S.; S. B. HAN, H. M. KIM, Y. H. KIM & J. J. LEE: 41-Demethylhomooligomycin B, a new immunosuppresant antibiotic from *Streptomyces ostreogriseus*. J. Antibiotics 49: 1275~1277, 1996
6) SAWADA, Y.; T. TSUNO, T. MIYAKI, T. NAITO & T. OKI: New cirramycin-family antibiotics F-1 and F-2: Selection of producer mutants, fermentation, isolation, structure elucidation and antibacterial activity. J. Antibiotics 42: 242~253, 1989
7) KAWAMURA, N.; R. SAWA, Y. TAKAHASHI, T. SAWA, N. KINOSHITA, H. NAGANAWA, M. HAMADA & T. TAKEUCHI: Amicenomycins A and B, new antibiotics from *Streptomyces* sp. MJ384-46F6. J. Antibiotics 48: 1521~1524, 1995