Notes

INOSTAMYCINS B AND C, NEW POLYETHER ANTIBIOTICS

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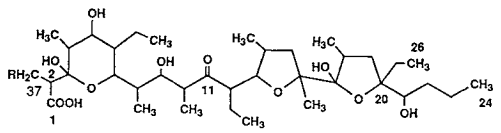
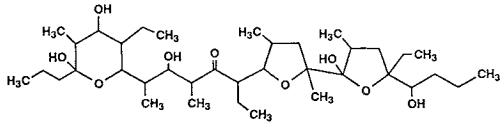
In the course of isolation of inostamycin (**1**), an inhibitor of phosphatidylinositol turnover, we found two related compounds, inostamycins B and C in the culture broth of *Streptomyces* sp. MH816-AF15.[1,2] In this paper, we report the production, isolation, physico-chemical properties, structures and biological properties of inostamycin B (**2**) and C (**3**) (Fig. 1). These compounds showed antimicrobial activity against Gram-positive bacteria *in vitro*, but were not inhibitory to phosphatidylinositol turnover.

The producing strain was cultured in 500-ml Erlenmeyer flasks containing 100 ml of glycerol 2.0%, dextrin 2.0%, soy peptone 1.0%, yeast extract 0.3%, $(NH_4)_2SO_4$ 0.2%, and $CaCO_3$ 0.02%, the pH being adjusted to 7.4 before sterilization. The fermentation was carried out for 5 days at 30°C on a rotary shaker (180 rpm/minute). The culture broth (25 liters) was centrifuged and the mycelium cake was extracted with acetone. The acetone extract was concentrated *in vacuo* and combined with the supernatant, then extracted with ethyl acetate. This extract was concentrated *in vacuo* and applied to a silica gel column which was developed with $CHCl_3$ and $CHCl_3$-MeOH (100:1). Combined fraction containing inostamycins was further purified by centrifugal partition chromatography (Sanki Engineering Limited, model LLN, solvent system: acetonitrile-hexane) to afford pure **1** (965 mg), **2** (29.8 mg) and **3** (38.3 mg).

Fig. 1. The structures of inostamycin, inostamycin B and C.



R=CH_3 Inostamycin
R=H Inostamycin B



Inostamycin C

Physico-chemical properties of **2** and **3** are summarized in Table 1. ^{13}C NMR chemical shifts of these antibiotics are shown in Table 2.

The molecular ion peak of **2** was observed in the FD-MS spectrum at *m/z* value 686, which is less than **1** by 14 mass units. Comparison of ^1H NMR spectra of **1** and **2** showed that one triplet methyl signal (38-H, δ_H 0.93) in **1** was replaced by a doublet methyl signal (37-H, δ_H 1.28) in **2**. Furthermore, a long range coupling was observed from the doublet methyl signal to the carboxyl group (C-1, δ_C 179.5) in the HMBC spectrum of **2**. Therefore, it was concluded that the ethyl group at C-2 in **1** was replaced to a methyl group in **2** (Fig. 1). Closely related compounds have recently been reported.[3]

The FD-MS spectrum of **3** gave a dehydration peak at *m/z* value 638. In the ^{13}C NMR spectra of **3**, one carboxyl group (C-1, δ_C 181.05) and one methine signal (C-2, δ_C 55.90) present in **1** was replaced by one methylene signal (C-2, δ_C 41.6). Thus, **3** was confirmed to be a decarboxyl compound of **1** (Fig. 1). Similar decarboxylation products were reported by KOENUMA *et al.*[4]

Table 1. Physico-chemical properties of inostamysins B and C.

	Inostamycin B		Inostamycin C	
MP	82~83°C		148~150°C	
$[\alpha]_D^{26}$ (*c* 0.5, $CHCl_3$)	+3.0		+2.6	
Molecular formula	$C_{37}H_{66}O_{11}$		$C_{37}H_{68}O_9$	
FD-MS	686 $(M)^+$		638 $(M-H_2O)^+$	
Analysis	Calcd:	Found:	Calcd:	Found:
C	64.68	63.88	67.63	68.02
H	9.69	9.36	10.44	10.13
Rf[a] in silica gel TLC	0.41		0.66	

[a] $CHCl_3$-MeOH (20:1).

Table 2. ^{13}C chemical shift assignment of inostamycins in $CDCl_3$.

	Inostamycin[a]	Inostamycin B	Inostamycin C		Inostamycin[a]	Inostamycin B	Inostamycin C
1 s	181.05	179.5		20 s	87.26	88.7	88.3
2-CH d	55.90	43.3		21 d	69.97	73.8	70.9
2-CH_2 t			41.6	22 t	34.82	33.6	34.0
3 s	100.69	100.6	100.2	23 t	20.28	19.4	19.4
4 d	38.22	38.6	38.9	24 q	14.40	14.1	14.1
5 d	71.17	71.6	71.5	25 t	31.11	30.8	30.4
6 d	37.57	36.8	36.6	26 q	7.18	7.5	7.4
7 d	74.75	75.8	75.1	27 q	14.74	12.1	13.7
8 d	32.30	33.1	32.8	28 q	23.98	24.2	24.3
9 d	76.50	78.0	78.1	29 q	15.56	14.2	15.0
10 d	47.48	48.2	47.5	30 t	14.99	14.6	16.5
11 s	214.96	210.8	213.3	31 q	12.54	12.7	12.5
12 d	55.16	54.1	55.2	32 q	12.84	13.5	13.7
13 d	83.62	82.3	84.2	33 q	5.28	4.6	4.7
14 d	34.73	33.9	35.2	34 t	18.41	20.0	18.9
15 t	42.68	42.1	42.0	35 q	10.85	11.2	10.8
16 s	86.29	82.9	83.9	36 q	13.17	13.1	13.7
17 s	108.30	107.6	107.4	37-CH_2 t	20.08		15.9
18 d	38.42	38.9	38.0	37-CH_3 q		12.8	
19 t	37.57	35.6	36.7	38 q	12.37		13.8

[a] Cited from the data by IMOTO *et al.*[1]

Table 3. Antimicrobial activities of inostamycins.

Test organism	MIC (μg/ml)		
	Inostamycin	Inostamycin B	Inostamycin C
Staphylococcus aureus FDA 209P	0.78	1.56	3.12
S. aureus Smith	0.78	3.12	100
S. aureus MS9610	0.78	3.12	100
S. aureus No. 5	0.78	3.12	100
S. aureus No. 17	0.78	6.25	>100
Micrococcus luteus FDA 16	0.78	3.12	12.5
M. luteus IFO 3333	0.78	3.12	12.5
M. luteus PCI 1001	0.78	3.12	>100
Bacillus anthracis	0.78	1.56	6.25
B. subtilis NRRL B-558	0.78	>100	>100
B. subtilis PCI 219	0.78	6.25	>100
B. subtilis ATCC 10702	0.78	1.56	6.25
Corynebacterium bovis 1810	0.78	3.12	6.25
Mycobacterium smegmatis ATCC 607	1.56	>100	>100
Escherichia coli NIHJ	>100	>100	>100
E. coli K-12	>100	>100	>100
Shigella dysenteriae JS 11910	>100	>100	>100
Salmonella typhi T-63	>100	>100	>100
Pseudomonas aeruginosa A3	100	>100	>100
Klebsiella pneumoniae PCT 602	>100	>100	>100
Candida albicans 3147	100	>100	>100

Mueller-Hinton agar, 37°C.

Inostamycins showed antimicrobial activities against Gram-positive bacteria. The results are given in Table 3. The antimicrobial activities of **2** and **3** are relatively reduced compared to those of **1**.

Inostamycins have cytocidal activity against *src*-NIH-3T3 cells. IC_{50} values of **1**, **2** and **3** were 0.07, 0.5 and 0.5 μg/ml, respectively.

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