NAPSAMYCINS, NEW *Pseudomonas* ACTIVE ANTIBIOTICS OF THE MUREIDOMYCIN FAMILY FROM *Streptomyces* sp. HIL Y-82,11372

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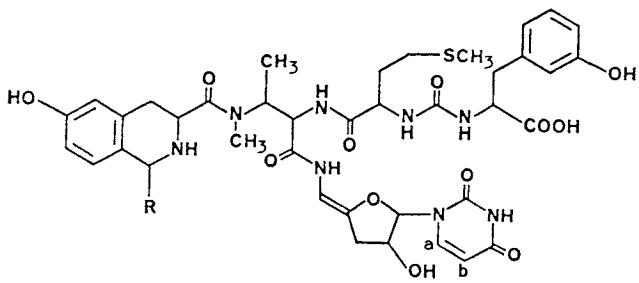
During the course of our screening program for antibiotics specifically active against *Pseudomonas*, we discovered four such new compounds which we named napsamycins A~D,[1] from the culture filtrate of a *Streptomyces* sp. HIL Y-82,11372 (DSM accession No. 5940). Chemical and spectroscopic analysis revealed that they belonged to the class of peptidylnucleoside antibiotics represented by the mureidomycins[2,3] and pacidamycins.[4,5] Herein we report the production, isolation, structural elucidation and biological activity of the napsamycins.

Strain HIL Y-82,11372 was isolated from a soil sample collected from the Andaman Islands, India. The strain was characterized as belonging to the genus *Streptomyces* by the methods described by SHIRLING and GOTTLIEB.[6] A loopful of mature slant culture of *Streptomyces* Y-82,11372 was inoculated into Erlenmeyer flasks (1-liter capacity) containing 150 ml of seed medium consisting of: soluble starch 2.4%, glucose 0.1%, beef extract 0.3%, tryptone 0.5%, soyabean meal 0.5%, yeast extract 0.5% and $CaCO_3$ 0.1%, pH 6.5 before autoclaving. The flasks were cultivated at 28°C on a rotary shaker at 220 rpm for 24 hours. The seed culture (4.5 liters) was inoculated into a 150-liter fermentor containing 90 liters of the production medium consisting of: soluble starch 2.0%, glucose 1.5%, soyatone 0.3%, peptone 0.3%, NaCl 0.2%, corn steep liquor 0.2%, $CaCO_3$ 0.2% and $(NH_4)_2SO_4$ 0.2%, pH 6.0 before autoclaving. The aeration and agitation of the fermentation were maintained at 80 and 100 rpm, respectively and the temperature was maintained at 28°C. Desmophen was added as the antifoam during a fermentation cycle of 68 hours. The production of the antibiotic was monitored by activity against *Pseudomonas aeruginosa* M35. The culture filtrate (150 liters) was passed through a column of Diaion HP-20 (7 liters) which was washed with demineralized water, 2 M aqueous NaCl, water, acidic water (pH adjusted to 3.5~4.0 with HCl), water and finally eluted with a stepwise gradient of 10% MeOH in water. Napsamycins eluted in 30~40% MeOH in water. This eluate was concentrated to dryness and chromatographed over silica gel (230~400 mesh, 1.4 kg) using a 5~10% stepwise gradient of 1% (v/v) ammoniacal MeOH in ethylacetate. Napsamycins eluted in 60:40 ethylacetate-ammoniacal MeOH. The active eluates were concentrated and further purified by medium pressure liquid chromatography (MPLC) over RP-18 (300 g, 60 μ) using a stepwise gradient of MeOH in water. Napsamycins eluted in 30% MeOH in water and this fraction was concentrated and lyophilized to give a mixture of

Fig. 1. Structures of napsamycins A~D.



Napsamycin A: R=H
Napsamycin B: R=CH_3
Napsamycin C: R=H; $\Delta^{a,b}$ reduced
Napsamycin D: R=CH_3; $\Delta^{a,b}$ reduced

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Table 1. Physico-chemical characteristics of napsamycins A~D.

	Napsamycin A	Napsamycin B	Napsamycin C	Napsamycin D
Appearance	White powder	White powder	White powder	White powder
Solubility	Aq. MeOH, DMSO and H_2O	Aq. MeOH, DMSO and H_2O	Aq. MeOH, DMSO and H_2O	Aq. MeOH, DMSO and H_2O
MP	>190°C (dec)	>190°C (dec)	>190°C (dec)	>190°C (dec)
Molecular weight	852	866	854	868
HRFAB-MS $(M+H)^+$				
Found:	853.3192	867.3347	855.3330	869.3504
Calcd:	853.3191 for $C_{39}H_{49}N_8O_{12}S$	867.3347 for $C_{40}H_{51}N_8O_{12}S$	855.3347 for $C_{39}H_{51}N_8O_{12}S$	869.3504 for $C_{40}H_{53}N_8O_{12}S$
Molecular formula	$C_{39}H_{48}N_8O_{12}S$	$C_{40}H_{50}N_8O_{12}S$	$C_{39}H_{50}N_8O_{12}S$	$C_{40}H_{52}N_8O_{12}S$
UV (H_2O) nm	254	256	254	256
(H_2O+1 N HCl) nm	254	256	254	256
(H_2O+1 N NaOH) nm	242, 294	240, 292	242, 294	240, 292
IR (KBr) cm^{-1}	3400~3300, 3120, 1690~1640	3395~3290, 3140, 1690~1645	3400~3300, 3110, 1690~1645	3400~3300, 3100, 1690~1650

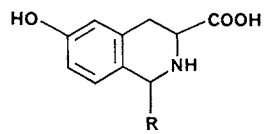
Table 2. ^1H NMR data of napsamycins A~D (400 MHz, 27°C, D_2O).

Moiety	Napsamycin A	Napsamycin B	Napsamycin C	Napsamycin D
m-Tyrosine				
	7.08 (t, 7.4 Hz, 1H)	7.12	7.07	7.10
	6.80 (dd, 8.8, 2.0 Hz, 1H)	6.79	6.83	6.81
	6.77 (br s, 1H)	6.71	6.73	6.73
	6.75 (br d, 8.8 Hz, 1H)	6.70	6.75	6.72
	4.50 (dd, 10, 4.5 Hz, 1H)	4.55	4.52	4.56
	2.93 (m, 2H)	2.83	2.99	2.85
Methionine				
	4.29 (m, 1H)	4.29	4.29	4.28
	2.49 (m, 2H)	2.50	2.48	2.51
	2.05 (s, 3H)	2.08	2.06	2.09
	1.92 (m, 2H)	1.94	1.93	1.98
AMBA				
	4.93 (dq, 8.8, 6.0 Hz, 1H)	4.91	4.90	4.94
	4.68 (d, 8.8 Hz, 1H)	4.68	4.71	4.70
	2.95 (s, 3H)	2.95	2.96	2.98
	1.21 (d, 6.0 Hz, 3H)	1.22	1.23	1.23
TIC				
	7.12 (d, 8.8 Hz, 1H)	7.18	7.15	7.15
	6.70 (br s, 1H)	6.66	6.69	6.65
	6.63 (dd, 8.8, 2.0 Hz, 1H)	6.58	6.61	6.59
	4.40 (d, 15 Hz, 1H)	4.65 (q, 5.9 Hz, 1H)	4.41	4.67
	4.36 (d, 15 Hz, 1H)	—	4.36	—
	4.28 (m, 1H)	4.25	4.29	4.24
	3.09 (m, 2H)	3.03, 1.62 (d, 5.9 Hz, 3H)	3.07	3.05, 1.60
Uracil/Dihydrouracil				
	7.34 (d, 7.3 Hz, 1H)	7.31	3.42 (m, 2H)	3.32
	5.63 (d, 7.3 Hz, 1H)	5.59	2.69 (m, 2H)	2.71
Sugar				
	6.12 (d, 1.0 Hz, 1H)	6.13	6.04	6.06
	6.01 (br s, 1H)	6.02	5.88	5.90
	4.57 (dd, 6, 1.5 Hz, 1H)	4.58	4.56	4.57
	2.80 (dd, 16.2, 9 Hz, 1H)	2.83	2.80	2.82
	2.69 (br d, 16.2 Hz, 1H)	2.72	2.75	2.71

napsamycins A ~ D as an off-white powder (0.568 g). Preparative HPLC on a 16 × 120 mm 10 μ ODS-Hypersil column using an eluant of 40 : 60 MeOH-0.02 M aq sodium phosphate buffer (pH 7.2) followed by desalting over HP-20 and elution with 50% MeOH in water gave mixtures of napsamycins A and C (65 mg) and napsamycins B and D (146 mg). Separation of the individual components was done by preparative HPLC on MN-Nucleosil $5C_{18}$ AB-ET column using a mixture of 91 : 9 H_2O-CH_3CN as eluant maintaining a flow rate of 0.8 ml/minute and UV detection at 254 nm. Concentration followed by lyophilization gave the pure napsamycin components as white powders.

The physico-chemical characteristics of the napsamycins are given in Table 1. Napsamycins C and D were the dihydro derivatives of components A and B, respectively. Acid hydrolysis (6 N HCl, 110°C, 18 hours) followed by GC-MS analysis of the hydrolysate as *N*-TFA-O-Me derivatives indicated the presence of *m*-tyrosine and 2-amino-3-*N*-methylaminobutyric acid (AMBA) in all the napsamycins. Surprisingly the presence of methionine could not be detected. Mild acid hydrolysis (2 N HCl, 90°C, 2 hours) furnished uracil from napsamycins A and B and dihydrouracil from C and D, respectively along with β-alanine, derived from the dihydrouracil moiety. The sugar unit could not be detected under such conditions presumably because of the instability of the exocyclic enol ether moiety. Drastic acid hydrolysis of components A and C also gave 6-hydroxy-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid (TIC) (**1**) and its 1-methyl analogue (**2**) from components B and D, respectively. The positions of the OH and the methyl groups in **1** and **2** were established by analysis of the ^1H NMR spectra of the napsamycins as revealed in Table 2. In general, the NMR spectra were complicated by many of the signals appearing in duplicate presumably because of slow conformational equilibria. Partial coalescence could be achieved by warming the solutions to 95°C which, however, led to line broadening.

The structures of the napsamycins were established by analysis of their FAB-MS/MS spectra using low-energy collision (33 eV) with argon (Fig. 2). Sequence selective fragmentations were observed for all the components showing complete similarity to those reported in the CID spectra of the mureidomycins A ~ D except those originating from the *N*-terminus. For both napsamycins A and C, the fragment ion *m/z* 148 was observed establishing that they had the same TIC unit as the blocked *N*-terminus. The corresponding fragments for the components B and D were at *m/z* 162, *i.e.* 14 mass units higher, confirming the presence of methyl substituted TIC unit at this site. The presence of the uracil units in A and B components was revealed by the fragment ion corresponding to a loss of *m/z* 112 from the molecular ion, whereas for C and D,



1: R = H
2: R = CH_3

Fig. 2. FAB-MS/MS fragmentations of napsamycins A ~ D.

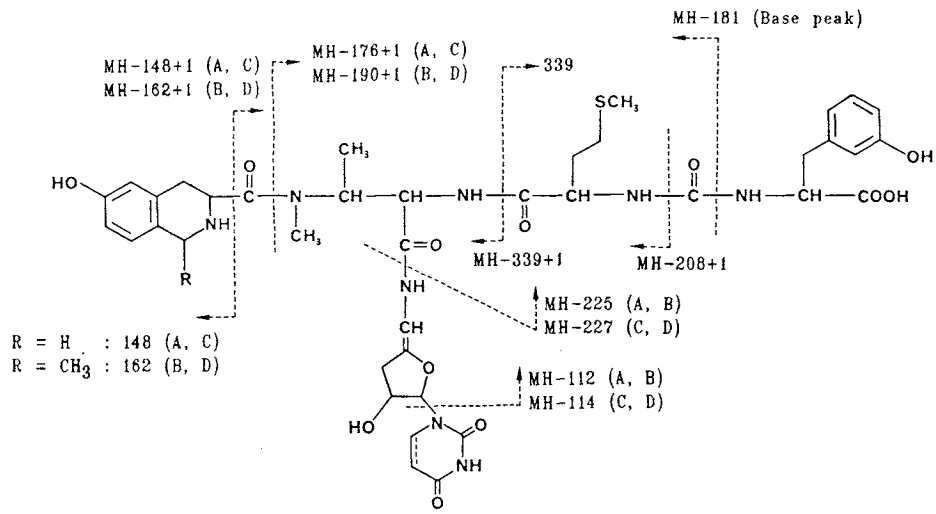


Table 3. Minimum inhibitory concentrations (MIC) of napsamycins A~D.

Test organism	MIC (mcg/ml)			
	Napsamycin A	Napsamycin B	Napsamycin C	Napsamycin D
Pseudomonas aeruginosa	25	12.5	25	12.5
P. aeruginosa 1771	50	25	50	25
P. aeruginosa 20653	50	25	25	25
P. aeruginosa M35	12.5	6.25	12.5	6.25
P. aeruginosa 80	50	25	50	25
P. aeruginosa NCTC 10701	25	25	25	25
P. fluorescens	100	50	100	50

loss of m/z 114 mass units confirmed the presence of dihydrouracil moieties. The structures of the napsamycins were thus established as those represented in Fig. 1. During the course of this work we came across a report[7] describing mureidomycins E and F which also possess a hydroxy-TIC unit at the *N*-terminus. It appears that napsamycin A is identical to mureidomycin F.

Biological Properties

Napsamycins A~D are active specifically against *Pseudomonas* strains. The MIC values are in the range of 6.25~100 mcg/ml for different strains (Table 3). Napsamycins A~D have very poor activity against other Gram-negative and Gram-positive bacteria, MIC values being higher than 100 mcg/ml.

References

1) GANGULI, B. N.; S. R. NADKARNI, M. V. PATEL, S. CHATTERJEE, J. BLUMBACH, H.-W. FEHLHABER & L. VERTESY: Napsamycins, new anti-pseudomonas antibiotics. Program and Abstracts of the 32nd Intersci. Conf. on Antimicrob. Agents Chemother., No. 504, p. 195, Anaheim, Oct. 11~14, 1992
2) INUKAI, M.; F. ISONO, S. TAKAHASHI, R. ENOKITA, Y. SAKAIDA & T. HANEISHI: Mureidomycins A~D, novel peptidylnucleoside antibiotics with spheroplast forming activity. I. Taxonomy, fermentation, isolation and physico-chemical properties. J. Antibiotics 42: 662~666, 1989
3) ISONO, F.; M. INUKAI, S. TAKAHASHI, T. HANEISHI, T. KINOSHITA & H. KUWANO: Mureidomycins A~D, novel peptidylnucleoside antibiotics with spheroplast forming activity. II. Structure elucidation. J. Antibiotics 42: 667~673, 1989
4) KARWOWSKI, J. P.; M. JACKSON, R. J. THERIAULT, R. H. CHEN, G. J. BARLOW & M. L. MAUS: Pacidamycins, a novel series of antibiotics with anti-*Pseudomonas aeruginosa* activity. I. Taxonomy, of the producing organism and fermentation. J. Antibiotics 42: 506~511, 1989
5) CHEN, R. H.; A. M. BUKO, D. N. WHITTERN & J. B. MCALPINE: Pacidamycins, a novel series of antibiotics with anti-*Pseudomonas aeruginosa* activity. II. Isolation and structure elucidation. J. Antibiotics 42: 512~520, 1989
6) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
7) ISONO, F.; Y. SAKAIDA, S. TAKAHASHI, T. KINOSHITA, T. NAKAMURA & M. INUKAI: Mureidomycins E and F, minor components of mureidomycins. J. Antibiotics 46: 1203~1207, 1993