8″-HYDROXYPACTAMYCIN AND 7-DEOXYPACTAMYCIN, NEW MEMBERS OF THE PACTAMYCIN GROUP

Sir:

8″-Hydroxypactamycin and 7-deoxypactamycin, new members of the pactamycin group of antibiotics[1~3], were found in culture filtrates of the microbial strain SIPI-A3-0121, which had been isolated by the Shanghai Institute of Pharmaceutical Industry and confirmed to belong to the genus *Streptomyces*. These antibiotics were isolated and their structures determined by NMR and MS studies. In this communication, the isolation, properties, structure, and biological activities of 8″-hydroxypactamycin and 7-deoxypactamycin are reported.

The slant culture of the producing organism was inoculated into a 500-ml conical flask containing 110 ml of a seed medium consisting of glucose 1.0%, glycerol 1.0%, potato starch 1.0%, corn steep liquor 1.0%, Polypepton 0.5%, yeast extract 0.2%, NaCl 0.1% and $CaCO_3$ 0.32% (adjusted to pH 7.4 before sterilization). After incubation at 27°C for 72 hours on a rotary shaker the culture broths of two flasks were combined and inoculated into a 30-liter jar fermentor containing 15 liters of a producing medium. This medium was composed of glycerol 2.5%, yeast extract 1.0%, meat extract 0.5%, Polypepton 0.5%, NaCl 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, K_2HPO_4 0.05% and $CaCO_3$ 0.32% (pH 7.4 before sterilization). The fermentation was carried out at 27°C for 68 hours under agitation of 200 rpm, with an aeration rate of 15 liters per minute.

The isolation and purification procedure are shown in Scheme 1. The fermentation broth (29.0 liters, pH 7.5) was adjusted to pH 3.3 with 5 M HCl and filtered. The active component was adsorbed onto 600 ml of Diaion HP-20 (Mitsubishi Chemical Industries Limited) and eluted with acetone - 0.05 M HCl (3 : 2). The fractions inhibiting the growth of *Bacillus subtilis* PCI 219 were collected and evaporated under reduced pressure to give an acetone-free concentrate. The active component was extracted with *n*-

Scheme 1.

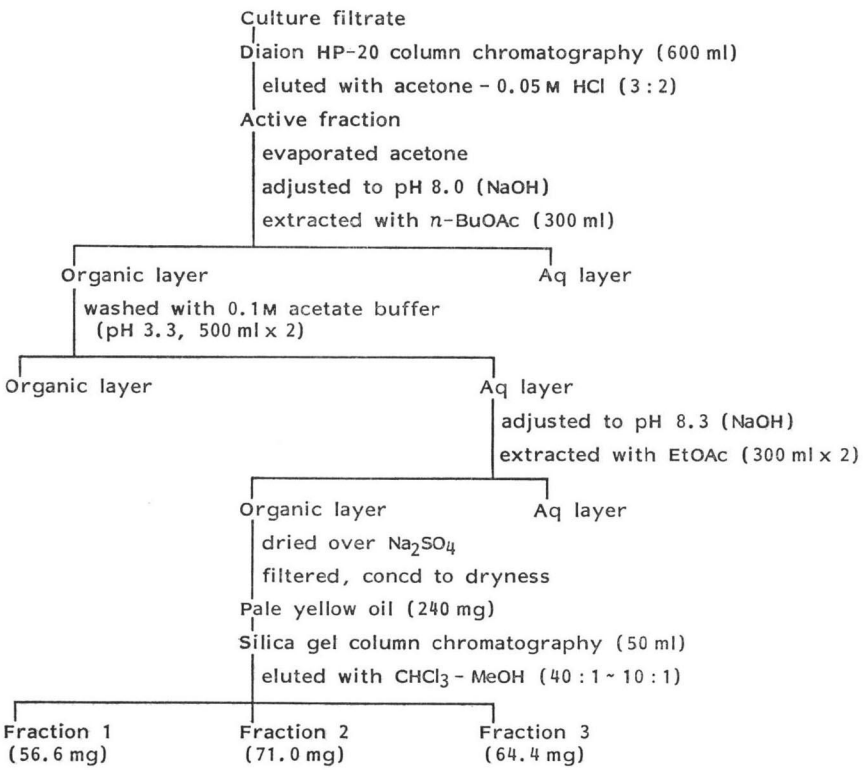


Table 1. Physico-chemical properties of pactamycin and related compounds.

	Pactamycin	A3-121B	A3-121C
MW	558	574	542
SI-MS (M+1)	559	575	543
$[\alpha]_D$	+23° (*c* 1.0, EtOH)[4]	+25.5° (*c* 0.5, EtOH) (29.0°C)	+39.1° (*c* 0.24, EtOH) (22.0°C)
UV λ_{max}	239 (a=52.6)[2], 264 (sh, 14.7), 313 (5.2), 356 (3.4)	210 (ε_{max}=40,100) 238 (40,700), 265 (sh, 10,400), 312 (3,970), 355 (2,260)	209 (ε_{max}=43,700) 236 (43,900), 264 (sh, 13,000), 310 (3,800), 355 (2,450)
HPLC[a] retention time (minutes)	16.07	9.85	14.22

[a] Column: SSC-N5C18 Nucleosil 5 micron, solvent: 50% MeOH - 0.01 M ammonium phosphate, pH 3.0, flow rate: 0.8 ml/minute, detection: UV (254 nm).

Table 2. Rf values of pactamycin, A3-121B and A3-121C.

	1-BuOH - AcOH - H_2O (10: 1: 1)	$CHCl_3$ - MeOH (10: 1)
Pactamycin	0.45	0.48
A3-121B	0.32	0.29
A3-121C	0.49	0.03

Table 3. ^{13}C NMR Chemical shifts of pactamycin and related compounds (100 MHz, $CDCl_3$).

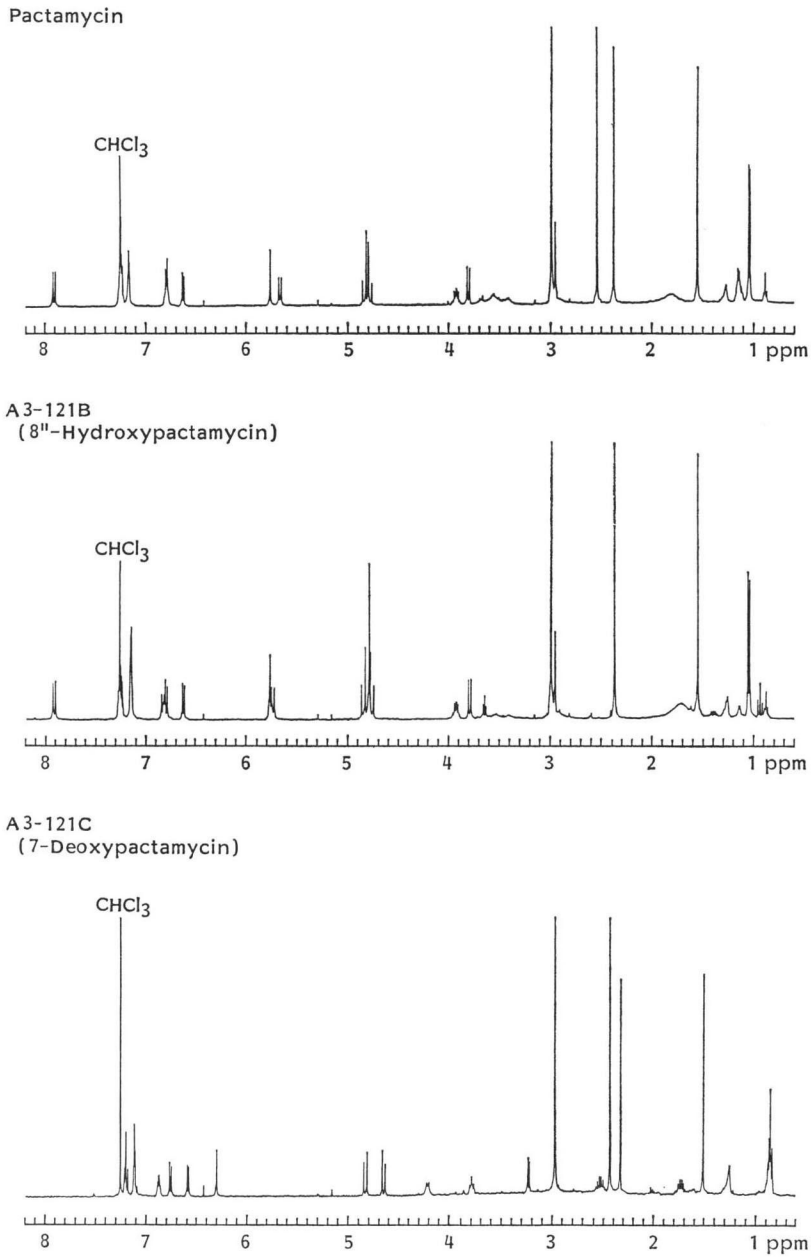
Position	Pactamycin	A3-121B	A3-121C
1	71.6	71.5	66.1
2	63.3	63.4	63.7
3	68.9	68.8	68.6
4	84.9	84.9	85.0
5	88.9	88.9	82.1
6 CH_3	21.1	21.1	21.7
7	74.3	74.3	24.9
8 CH_3	18.0	18.0	8.4
9 CH_2	65.4	65.4	65.4
10 C=O	159.3	159.2	158.4
11,12 $CH_3\times2$	36.9	36.9	36.5
1′	112.2	112.0	112.8
2′	162.7	162.7	161.7
3′	115.7	115.7	115.6
4′	134.5	134.5	134.1
5′	123.0	123.0	122.8
6′	141.2	141.1	141.2
7′ CH_3	24.0	24.0	23.4
8′ C=O	172.4	172.4	170.8
1″	138.4	134.6	138.0
2″	110.9	110.2	112.0
3″	146.6	146.9	147.6
4″	118.7	119.7	118.3
5″	129.6	130.0	129.4
6″	118.4	117.0	117.8
7″ C=O	198.4	198.6	198.2
8″	26.6	65.3	26.5

Chemical shifts are given downfield from TMS.

butyl acetate at pH 8.0. It was further purified by extraction with 0.1 M acetate buffer at pH 3.3, followed by extraction with ethyl acetate at pH 8.3. The evaporation of the solvent gave a pale yellow oil (240 mg), which was then applied onto a silica gel column and eluted with chloroform - methanol. The early antibiotic-containing active fraction (fraction 1) was further purified by silica gel preparative thin-layer chromatography using 1-BuOH - acetic acid - water mixture (10: 1: 1) to yield two antibiotics, A3-121A (8.4 mg) and A3-121B (1.1 mg). The oil obtained from fraction 2 was purified by preparative TLC to give A3-121B (3.4 mg). Fraction 3 gave A3-121C (4.5 mg) by the same procedure. A3-121A was identified as pactamycin from its UV, IR, ^1H NMR and ^{13}C NMR spectra[2,4,5].

Physico-chemical properties of pactamycin, A3-121B and A3-121C are summarized in Table 1. These three antibiotics were soluble in MeOH, EtOH, $CHCl_3$ and CH_2Cl_2, but insoluble in water and *n*-hexane. The hydrogen chloride salts of these three antibiotics were soluble in water. These antibiotics gave positive color reactions to ninhydrin, Rydon-Smith and 2,4-dinitrophenylhydrazine reagents. Secondary ion mass spectra (SI-MS) of pactamycin, A3-121B and A3-121C gave their (M+1) peaks at *m/z* 559, 575 and 543, respectively. As shown in Table 1, the UV spectra of A3-121B and A3-121C are very similar to that of pacta-

Fig. 1. ^{1}H NMR spectra of pactamycin, A3-121B and A3-121C (400 MHz, $CDCl_3$).



mycin. The Rf values of pactamycin, A3-121B and A3-121C on silica gel TLC are shown in Table 2.

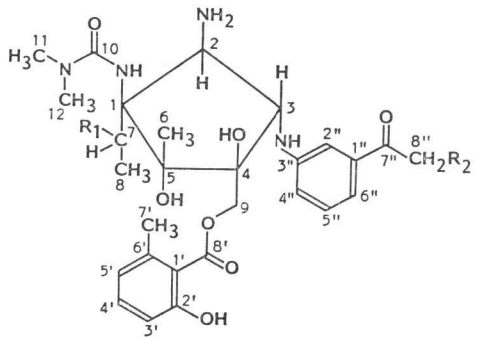
Chemical shifts in ^{13}C NMR spectra of pactamycin, A3-121B and A3-121C in $CDCl_3$ solution were assigned as shown in Table 3. Our assignments were in good agreement with those reported by WELLER *et al.*[5].

The 400 MHz ^{1}H NMR spectra of these three antibiotics in $CDCl_3$ are shown in Fig. 1. The assignments of these spectra were made from ^{1}H-^{1}H-correlated spectroscopy (COSY) spectra. In the spectrum of pactamycin, there were six methyl signals, which were assigned to H-8 [1.03 ppm, d (J=6.6 Hz), 3H], H-6 (1.55 ppm, s, 3H), H-7′ (2.38 ppm, s, 3H), H-8″ (2.55 ppm,

Table 4. The antimicrobial spectra of pactamycin, 8″-hydroxypactamycin and 7-deoxypactamycin.

Test organism	Pactamycin	8″-Hydroxy-pactamycin	7-Deoxy-pactamycin
Staphylococcus aureus FDA 209P	0.1	0.2	0.2
S. aureus Smith	0.39	0.39	0.39
Micrococcus luteus PCI 1001	0.1	0.1	0.025
Bacillus anthracis	50	50	25
B. subtilis PCI 219	0.39	0.2	0.1
B. subtilis NRRL B-558	0.1	0.2	0.1
Escherichia coli NIHJ	1.56	1.56	1.56
E. coli K-12	25	12.5	25
E. coli K-12 ML 1629	25	12.5	25
Shigella dysenteriae JS 11910	1.56	1.56	0.78
S. flexneri 4b JS 11811	6.25	6.25	12.5
S. sonnei JS 11746	12.5	6.25	12.5
Salmonella typhi T-63	50	12.5	50
S. enteritidis 1891	3.12	1.56	1.56
Proteus vulgaris OX 19	12.5	6.25	12.5
Serratia marcescens	25	25	50
Klebsiella pneumoniae PCI 602	0.78	0.2	0.2
Pseudomonas aeruginosa A3	12.5	6.25	6.25
Mycobacterium smegmatis ATCC 607	>100	50	6.25

Fig. 2. Structures of A3-121B, A3-121C and pactamycin.



Pactamycin	R_1=OH	R_2=H
A3-121B (8″-Hydroxypactamycin)	R_1=OH	R_2=OH
A3-121C (7-Deoxypactamycin)	R_1=H	R_2=H

s, 3H) and H-11,12 (2.98 ppm, s, 6H). A methylene signal for H-9 [4.76 ppm, 4.85 ppm, AB (J=11.8 Hz), 2H] and three methine signals, one each for H-2 (2.95 ppm, s, 1H), H-3 [3.80 ppm, d (J=11.0 Hz), 1H] and H-7 [3.92 ppm, dq ($J_{7,8}$=6.6 Hz, $J_{7,OH}$=11.8 Hz), 1H], were also assigned.

The ^{1}H NMR spectrum of A3-121B showed five methyl signals [1.05 ppm, d (J=6.4 Hz), 3H], (1.56 ppm, s, 3H), (2.37 ppm, s, 3H), (2.99 ppm, s, 6H). A singlet signal at 4.80 ppm (2H), which was not present in the spectrum of pactamycin, was assigned to a methylene signal, $COCH_2OH$. Other signals (methylene and methine and aromatic methine signals) were the same as those of pactamycin. Therefore, the structure shown in Fig. 2 was proposed for A3-121B, where the methyl group at C-8″ of pactamycin is replaced by a hydroxymethyl group. The presence of a new methylene peak was detected at 65.3 ppm in the ^{13}C NMR spectra of 8″-hydroxypactamycin instead of the methyl signal at 26.6 ppm in pactamycin and was confirmed as follows; the methylene signal at 65.3 ppm was differentiated from the methylene signal at 65.4 ppm by comparison of the gated decoupling spectrum and the low power selective proton decoupled (LSPD) spectrum irradiating at 3.73 ppm (H-3). Assignments of methine peaks at 84.9 ppm (C-4) and 88.9 ppm (C-5) were also confirmed by LSPD, irradiating at 1.55 ppm (6-CH_3) and 3.73 ppm (H-3).

The ^{1}H NMR spectrum of A3-121C showed the following six methyl signals: H-8 [0.87 ppm, t (J=7.2 Hz), 3H], H-6 (1.51 ppm, s, 3H), H-7′ (2.32 ppm, s, 3H), H-8″ (2.41 ppm, s, 3H) and H-11,12 (2.96 ppm, s, 6H). There were signals of a non-equivalent methylene system at 1.88 ppm (m, 1H) and 2.43 ppm (m, 1H) that were correlated to the H-8 methyl signal in the COSY spectrum. Thus the spectrum indicated

that the structure of A3-121C should be 7-deoxypactamycin. This structure was confirmed by a comparison of the ^{13}C NMR spectrum of A3-121C with that of pactamycin: the presence of a new methylene signal at 24.9 ppm, a large upfield shift of C-8 (18.0 ppm→8.4 ppm) and C-1 (71.6 ppm→66.1 ppm), and the absence of the methine signal at 74.3 ppm seen in pactamycin. Antibiotic T-47811, which was reported by ASAI and co-workers in 1979[6], seems to be identical with 7-deoxypactamycin from its IR and ^1H NMR spectra.

The antimicrobial activities of 8″-hydroxypactamycin and 7-deoxypactamycin as well as pactamycin were measured by the agar dilution method (Table 4). All three antibiotics inhibited the growth of Gram-positive and Gram-negative bacteria. The 50% growth inhibitory concentrations (IC_{50}) against L1210 cells were 0.027 μg/ml for 8″-hydroxypactamycin and 0.026 μg/ml for 7-deoxypactamycin. The toxicities (LD_{100}, ip) of 8″-hydroxypactamycin and 7-deoxypactamycin in mice were 25 mg/kg and 1.57 mg/kg, respectively.

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