FOSFADECIN AND FOSFOCYTOCIN†, NEW NUCLEOTIDE ANTIBIOTICS PRODUCED BY BACTERIA

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Two new nucleotide antibiotics, fosfadecin and fosfocytocin, have been isolated from the culture filtrates of *Pseudomonas viridiflava* PK-5 and *Pseudomonas fluorescens* PK-52, respectively. These antibiotics were purified by column chromatographies using adsorption, gel filtration and ion exchange resins. On the basis of the spectroscopic and degradation studies, the chemical structures of fosfadecin and fosfocytocin were determined. These antibiotics were either enzymatically or chemically hydrolyzed to generate fosfomycin and a new antibiotic, fosfoxacin, which are also produced in the culture filtrates. They showed antibacterial activity against Gram-positive and Gram-negative bacteria. The antibacterial activity of these nucleotide antibiotics was weaker than that of fosfomycin and fosfoxacin.

In the course of screening for new antibiotics which inhibit bacterial cell wall biosynthesis, we found that new nucleotide antibiotics, fosfadecin (**1**) and fosfocytocin (**2**) were produced by Gram-negative bacteria. These two antibiotics were converted to their nucleoside 5'-monophosphates (**5** and **6**, respectively), in addition, fosfadecin yielded fosfomycin[1] (**3**) and fosfocytocin yielded a new antibiotic, fosfoxacin (**4**) (Fig. 1).

The present paper describes the producing organisms, fermentation, isolation, structure determination and biological activity of these nucleotide antibiotics.

Producing Organism

The fosfadecin-producing strain PK-5 was isolated from a bamboo leaf collected in Yumesaki-cho, Shikama-gun, Hyogo Prefecture, Japan. The fosfocytocin-producing strain PK-52 was isolated from a soil sample collected in Shimotsu-cho, Kaiso-gun, Wakayama Prefecture, Japan.

The taxonomical characteristics of strains PK-5 and PK-52 are summarized in Table 1.

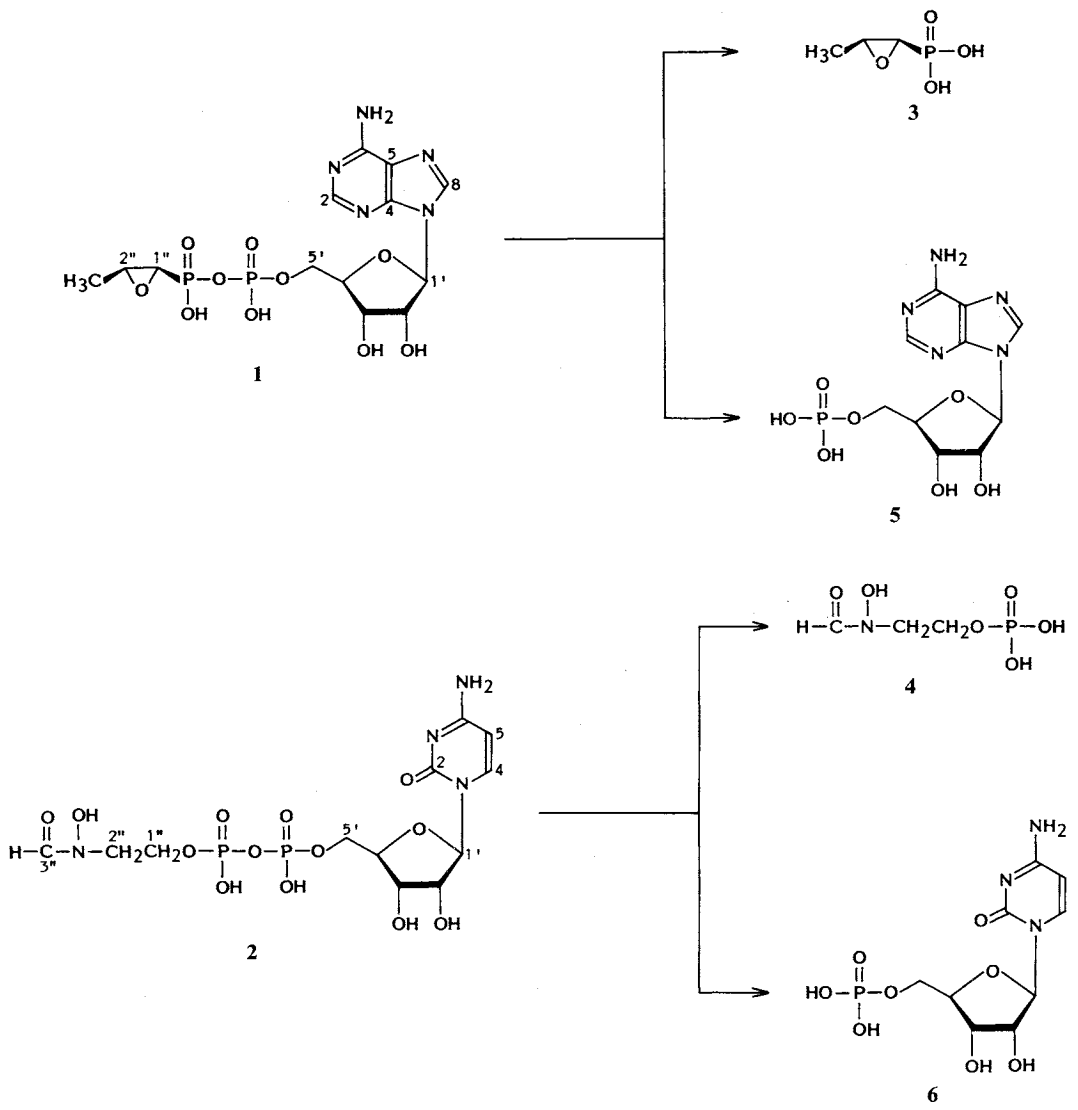
Strains PK-5 and PK-52 are Gram-negative rods, motile by polar flagella, metabolize glucose oxidatively, and are catalase-positive. The mol % G+C of the DNA is 65.1 and 63.7, respectively. Strains PK-5 and PK-52 are considered to belong to the genus *Pseudomonas*.

Strain PK-5 does not accumulate poly-β-hydroxybutyrate as a carbon reserve material, and it produces fluorescent pigment. This strain is also negative for arginine dihydrolase and oxidase. These properties indicate that it should be classified as *Pseudomonas syringae* or *Pseudomonas viridiflava*. As strain PK-5 has a polar flagellum, it was identified as *P. viridiflava* and designated *P. viridiflava* PK-5.

Strain PK-52 does not accumulate poly-β-hydroxybutyrate and produces fluorescent pigment. It has more than one flagella. It is arginine dihydrolase positive and hydrolyzes gelatin. From these characteristics,

† Fosfadecin was presented as TAN-930 in Jpn. Kokai 170395 ('88), July 14, 1988, and fosfocytocin was presented as TAN-1022 in Jpn. Kokai 168695 ('89), July 4, 1989.

Fig. 1. Degradation studies of fosfadecin (**1**) and fosfocytocin (**2**).



strain PK-52 was identified as *P. fluorescens* and designated *P. fluorescens* PK-52.

P. viridiflava PK-5 and *P. fluorescens* PK-52 have been deposited in the Institute for Fermentation, Osaka and have the accession No. IFO 14515 and IFO 14676, respectively.

Fermentation

Fosfadecin (**1**) and Fosfomycin (**3**)

A loopful of *P. viridiflava* PK-5 cells which had been grown on nutrient agar was inoculated into a 2-liter Sakaguchi flask containing 500 ml of seed medium consisting of glucose 2%, soluble starch 3%, corn steep liquor 0.3%, Polypepton (Nihon Pharmaceutical Co., Ltd.) 0.5%, NaCl 0.3%, $CaCO_3$ 0.5%, (pH 7.0). The cultivation was carried out at 24°C for 48 hours on a reciprocal shaker. The seed culture thus obtained was transferred to a 50-liter fermenter containing 30 liters of the same seed medium

Table 1. Morphological and physiological characteristics of strains PK-5 and PK-52.

Characteristic	PK-5	PK-52	Characteristic	PK-5	PK-52
Gram stain	Negative	Negative	Catalase	+	+
Size (μm): width	0.8~1.0	0.4~1.0	Oxidase	−	+
length	1.0~2.0	0.9~2.0	Range of growth		
No. of flagella	1	>1	pH	5.2~8.8	5.0~9.5
Motility	+	+	Optimal pH	5.3~5.9	5.0~8.0
Spore formation	−	−	Temperature (°C)	15~35	15~30
O-F test	Oxidative	Oxidative	Optimal temperature (°C)	23~29	17~24
Reduction of nitrate	−	−	Oxygen demand	Aerobic	Aerobic
Denitrification	−	−	Degradation of Tween 80	+	+
Methyl red test	−	−	Liquefaction of gelatin	+	+
Voges-Proskauer test	−	−	Accumulation of poly-β-hydroxybutyrate	−	−
Production of hydrogen sulfide	−	−			
Hydrolysis of starch	−	−	Fluorescent pigment	+	+
Utilization of citrate	+	+	Arginine dihydrolase	−	+
Utilization of potassium nitrate	−	−	Arginine decarboxylase	−	+
ammonium sulfate	−	−	Mol % of G+C of DNA	65.1	63.7
Urease	+	+			

supplemented with 0.05% Actcol (Takeda Chem. Ind., Ltd.), an antifoaming agent. The cultivation was carried out at 24°C for 48 hours with an agitation of 200 rpm and an aeration of 30 liters per minute. Six liters of this culture was transferred to a 200-liter fermenter containing 120 liters of a fermentation medium consisting of glucose 3%, yeast extract 0.25%, NZ-amine A 1.0%, and Actcol 0.05%. The fermentation was carried out at 17°C for 66 hours with an agitation of 150 rpm and an aeration of 200 liters per minute. Fosfadecin was detected starting in the early stage of the cultivation and fosfomycin was detected later and in increasing amounts with time. The titer of fosfadecin plus fosfomycin calculated as fosfadecin was about 50 μg/ml under these conditions.

Fosfocytocin (**2**) and Fosfoxacin (**4**)

P. fluorescens PK-52 was cultivated in the same manner as that described for fosfadecin, except that the fermentation medium consisted of sucrose 3%, corn steep liquor 1.5%, dried yeast 1.5%, and Actcol 0.05% (pH 6.5). Fosfocytocin was detected from the early stage to the end of fermentation, while fosfoxacin was produced in later stage. Unlike fosfomycin, fosfoxacin did not accumulate to a great extent. The titer of fosfocytocin plus fosfoxacin calculated as fosfocytocin was about 60 μg/ml.

Isolation

Fosfadecin (**1**)

The culture broth of strain PK-5 was filtered through Hyflo-Super Cel. The filtrate (98 liters) was applied to a column of Amberlite IRA-402 (Cl^- type, 5 liters), and the active substance was eluted with 1 M NaCl (50 liters). The eluate was subjected to activated carbon chromatography (4 liters) eluting with 8% isobutyl alcohol-0.02 M ammonia (16 liters). The eluate was concentrated and chromatographed on QAE-Sephadex A-25 (Cl^- type, 3 liters) eluting with 0.1 M NaCl. The active fractions were combined and subjected to activated carbon chromatography (0.8 liters) eluting with 8% isobutyl alcohol. The eluate was concentrated and again chromatographed on QAE-Sephadex A-25 (Cl^- type, 1 liter), this time eluting with 0.09 M NaCl. The active fractions were desalted with activated carbon (150 ml), concentrated and freeze-dried to give a white powder of fosfadecin as the disodium salt (1.46 g).

Fosfomycin (3)

The culture broth of strain PK-5 was centrifuged and the supernatant (700 ml) was chromatographed on a column of Dowex 1X2 (50~100 mesh, Cl^- type, 35 ml) eluting with 2% NaCl. After being adjusted to pH 5, the eluate was purified by alumina chromatography (20 ml). The medium was washed with water, 75% methanol and 75% methanol - 1 M ammonia, and active substance was finally eluted with 1 M ammonia water. The eluate was concentrated and methanol (60 ml) was added to the concentrate (6 ml). The mixture was filtered and the insoluble material was discarded. The filtrate was concentrated and freeze-dried to give a crude powder of fosfomycin (195 mg, purity 5%).

Fosfocytocin (2)

The culture broth of strain PK-52 was filtered through Hyflo-Super Cel. The filtrate (100 liters) was applied to a column of Amberlite IRA-402 (Cl^- type, 7 liters), and the active substance was eluted with 1 M NaCl (30 liters). The eluate was subjected to activated carbon chromatography (10 liters) eluting with 8% isobutyl alcohol (50 liters). The eluate was concentrated and chromatographed on Amberlite IRA-68 (Cl^- type, 2 liters) eluting with 1 M NaCl. The active fractions were desalted with activated carbon (2 liters) and concentrated. The concentrate was chromatographed on QAE-Sephadex A-25 (Cl^- type, 2.2 liters) eluting with 0.1 M NaCl. The active fractions were desalted with activated carbon, concentrated and freeze-dried to give a crude powder of fosfocytocin (1.72 g). The powder (870 mg) was purified by preparative HPLC using a YMC-Pack SH-343 (Yamamura Chem. Lab.) with a mobile phase of 0.02 M phosphate buffer (pH 6.3). The pure fractions were desalted with activated carbon, concentrated and freeze-dried to give a white powder of fosfocytocin as the disodium salt (295 mg).

Fosfoxacin (4)

The culture broth of strain PK-52 was centrifuged and the supernatant (1.5 liters) was passed through a column of activated carbon (150 ml). The effluent was chromatographed on a column of Dowex 1X2 (50~100 mesh, Cl^- type, 150 ml) eluting with 1 M NaCl. After being adjusted to pH 2.0, the eluate was chromatographed on a column of activated carbon (150 ml) eluting with water. The active fractions were concentrated and adjusted to pH 2.0. The concentrate was again chromatographed on activated carbon (100 ml), this time eluting with 8% isobutyl alcohol. The active fractions were concentrated and freeze-dried to give a crude powder of fosfoxacin (47 mg, purity 3%).

Physico-chemical Properties

The physico-chemical properties of fosfadecin (**1**), fosfocytocin (**2**) and fosfoxacin (**4**) are summarized in Table 2. They are soluble in water and dimethyl sulfoxide, and sparingly soluble in acetone and ethyl acetate. The molecular formulae of **1**, **2** and **4** were determined based on the elemental analyses, the molecular ion peaks in the SI-MS and the carbon numbers in the ^{13}C NMR spectra. The UV absorption spectra suggested **1** had an adenine chromophore and **2** had a cytosine chromophore. Compounds **1**, **2** and **4** showed a positive color reaction to molybdate reagent suggesting the presence of a phosphoric acid function. Furthermore, compounds **2** and **4** showed a positive color reaction to ferric chloride suggesting the presence of a hydroxyamide moiety.

Structural Determination

The ^{13}C, ^{1}H and ^{31}P NMR data are summarized in Tables 3 and 4. When **1** was hydrolyzed in 1 N

Table 2. Physico-chemical properties of fosfadecin (**1**), fosfocytocin (**2**) and fosfoxacin (**4**).

Property	1	2	4
Appearance	White powder	White powder	White powder
$[\alpha]_D^{23}$	$-26°$ (c 0.51, H_2O)	$+9.4°$ (c 0.50, H_2O)	
SI-MS (m/z)[a] $(M+H)^+$	512	535	208
Molecular formula	$C_{13}H_{17}N_5O_{10}P_2Na_2$ $(2H_2O)$	$C_{12}H_{18}N_4O_{13}P_2Na_2$ $(1\frac{1}{2}H_2O)$	$C_3H_7NO_6PNa$ $(2H_2O)$
Anal	Calcd Found	Calcd Found	Calcd Found
	C 28.53 28.63	C 25.68 26.14	C 14.82 14.78
	H 3.87 3.93	H 3.77 4.18	H 4.56 4.19
	N 12.80 12.96	N 9.98 9.67	N 5.76 5.63
	P 11.32 11.56	P 11.04 10.31	P 12.74 12.72
UV λ_{max} nm (ε)	H_2O: 258 (16,100) OH^-: 258 (16,300) H^+: 256 (15,900)	H_2O: 270 (9,300) OH^-: 268 (9,500) H^+: 278 (12,900)	End absorption
IR ν_{max} (KBr) cm^{-1}	3400, 1650, 1610, 1480, 1420, 1340, 1240, 1110, 1080, 940	3430, 1660, 1490, 1250, 1110, 1080, 940	3150, 1670, 1400, 1160, 1060, 940
Color reaction			
Positive	Molybdate reagent, Periodate-benzidine	Moylbdate reagent, Periodate-benzidine, $FeCl_3$,	Molybdate reagent, $FeCl_3$
Negative	Ninhydrine, $FeCl_3$	Ninhydrine	Periodate-benzidine, Ninhydrine

[a] The SI-MS were measured with a Hitachi M-80A mass spectrometer.

sodium hydroxide, adenosine 5'-monophosphate (**5**) and fosfomycin (**3**) were obtained as shown in Fig. 1. In the ^{31}P NMR spectrum of **1**, two signals coupled with each other. This data showed that **3** and **5** were bound by a phosphate bond.

Upon enzymatic hydrolysis, **2** gave cytidine 5'-monophosphate (**6**) and **4**. And the same as with **1**, signals coupled in the ^{31}P NMR spectrum of **2**. Therefore **4** and **6** are bound by a phosphate bond.

The ^1H NMR spectrum of **4** showed a formyl proton at 7.97 and 8.35 ppm and two methylene protons at 3.76 and 4.04 ppm. In the ^{13}C NMR spectrum of **4**, each signal was observed as a pair. This was explained by the existence of conformational isomers of the formyl amide moiety. Furthermore, in the proton decoupled ^{13}C NMR spectrum, two methylene signals coupled with phosphorus ($J=5.1$ and 7.0 Hz). From all these results, the structure of **4** was determined to be 2-(*N*-formyl-*N*-hydroxy)aminoethyl phosphoric acid.

Biological Activity

The antibacterial activity of fosfadecin, fosfomycin, fosfocytocin and fosfoxacin is shown in Table 5. Fosfadecin was more active against the fosfomycin-sensitive mutants (EF-7, PF-4) than against their parents (LD-2, IFO 3080). Fosfocytocin and fosfoxacin showed higher antibacterial activity against the fosmidomycin[2]-sensitive mutant (NCWP) than against the parent (NCW). Fosfoxacin exhibited potent antibacterial activity against *Bacillus cereus*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Aeromonas caviae*.

The nucleotide antibiotics, fosfadecin and fosfocytocin, showed weaker antibacterial activity than fosfomycin and fosfoxacin, which do not have the nucleotide moiety.

All of these antibiotics induced spheroplast formation in Gram-negative bacteria in a hypertonic medium (data not shown), suggesting that they inhibit biosynthesis of bacterial cell wall.

Fosfadecin was effective in mice against experimental infection with *Staphylococcus aureus* and

Table 3. ^{13}C and ^{1}H NMR spectral data of fosfadecin (**1**), fosfocytocin (**2**) and fosfoxacin (**4**) in D_2O.[a]

Position	^{13}C NMR			^{1}H NMR	
	δ (ppm)	Complete decoupling[b]	Off resonance	δ (ppm)	m[b]
1:					
6	158.34	s	s		
2	155.68	s	d	8.27	s
4	151.86	s	s		
8	142.67	s	d	8.51	s
5	121.39	s	s		
1′	89.82	s	d	6.15	d, J=5.6
4′	86.67	d, J=8.5	dd	4.41	m
2′	77.09	s	d	4.78	t, J=5.6
3′	73.23	s	d	4.55	dd, J=3.5, 5.6
5′	68.02	d, J=5.5	dt	4.23	dd, J=3.3, 5.0
2″	56.95	s	d	3.33	m
1″	55.47	d, J=195.9	dd	3.15	dd, J=5.0, 24.8
3″	16.39	s	q	1.50	d, J=5,4
2:					
2	167.65	s	s		
3″	163.23, 167.16	s	d	7.97, 8.34	s
6	158.83	s	s		
4	144.62	s	d	8.00	d, J=7.6
5	99.05	s	d	6.14	d, J=7.6
1′	92.01	s	d	5.99	d, J=4.1
4′	85.46	d, J=8.5	dd	4.10~4.40	m
2′	76.92	s	d	4.10~4.40	m
3′	71.98	s	d	4.10~4.40	m
5′	67.35	d, J=5.1	dt	4.10~4.40	m
1″	63.94, 64.50	d, J=5.4	dt	4.10~4.40	m
2″	49.55, 53.78	d, J=7.5	dt	3.78	m
4:					
3″	163.23, 167.20	s	d	7.97, 8.35	s
1″	63.01, 63.35	d, J=5.1	dt	4.04	m
2″	49.79, 54.02	d, J=7.0	dt	3.76	m

[a] The δ values were recorded in ppm downfield from 3-(trimethylsilyl)propionic acid-d_4 sodium salt using a Bruker AC-300.

[b] m: Multiplicity. Coupling constants are given in Hz.

Escherichia coli. The ED_{50} value is shown in Table 6. Fosfocytocin and fosfoxacin were not effective in experimental therapy.

Table 4. ^{31}P NMR spectral data of fosfadecin (**1**), fosfocytocin (**2**) and fosfoxacin (**4**) in D_2O.

Compound	δ (ppm)	Complete decoupling[a]
1	4.86	d, J=25.6
	−11.24	d, J=25.6
2	−10.88, −11.01	d, J=20.9
	−11.30, −11.39	d, J=20.9
4	0.61	br s

[a] Coupling constants are given in Hz.
The δ values referenced to phosphoric acid as external standard at 0 ppm.

Discussion

During the search for new antibiotics which inhibit bacterial cell wall biosynthesis, biological activity similar to that of fosfomycin was detected in the culture filtrate of bacteria. The active principle was identified as fosfomycin and new antibiotics fosfadecin, fosfocytocin and fosfoxacin. It has been reported that *P. syringae* PB-5123 produces fosfomycin in its culture filtrate[3]. *P. viridiflava* PK-5 seems to differ from *P. syringae* PB-5123, because the latter has multitricous flagella and produces arginine dihydrolase.

The structure of fosfadecin was confirmed to be a combination of the structures of fosfomycin and

Table 5. Antibacterial activities of fosfadecin, fosfocytocin and fosfoxacin.

Test organism	MIC (μg/ml)			
	Fosfadecin	Fosfomycin	Fosfocytocin	Fosfoxacin
Staphylococcus aureus FDA 209P	>100 (6.25)[a]	12.5	>100	>100
S. epidermidis IFO 3762	>100 (>100)	50	>100	>100
Bacillus subtilis NIHJ PCI 219	>100 (>100)	6.25	>100	50
B. cereus FDA 5	>100 (>100)	3.13	100	0.2
Micrococcus luteus IFO 12708	>100 (100)	>100	100	0.2
Escherichia coli NIHJ JC-2	>100 (3.13)	6.25	100	>100
E. coli LD-2	>100 (0.78)	12.5	>100	>100
E. coli EF-7	25	0.1	>100	>100
Pseudomonas aeruginosa IFO 3080	>100 (>100)	6.25	>100	1.56
P. aeruginosa PF-4	>100 (50)	0.39	>100	1.56
Aeromonas caviae NCW	>100 (6.25)	0.78	>100	1.56
A. caviae NCWP	NT	NT	25	0.1
A. caviae NCWO	>100 (>100)	>100	>100	>100
Salmonella typhimurium IFO 12529	>100 (6.25)	12.5	>100	>100
Citrobacter freundii IFO 12681	>100 (25)	6.25	>100	>100
Klebsiella pneumoniae IFO 3317	>100 (50)	100	>100	>100
Enterobacter cloacae IFO 12937	>100 (100)	100	>100	>100
Serratia marcescens IFO 12648	>100 (12.5)	1.56	>100	>100
Proteus mirabilis ATCC 21100	>100 (3.13)	3.13	>100	>100
P. vulgaris IFO 3988	>100 (>100)	25	>100	>100

MICs were determined by the microtiter plate method using Bacto-Nutrient Broth (Difco) containing 0.002% diaminopimelic acid.

Final inoculum size was 10^5 cfu/ml.

[a] Figures in parentheses mean MAC (μg/ml), maximum allowance concentrations of growth.

E. coli EF-7: Fosfomycin-sensitive mutant derived from LD-2. *P. aeruginosa* PF-4: fosfomycin-sensitive mutant derived from IFO 3080. *A. caviae* NCWP: fosmidomycin-sensitive mutant derived from NCW. *A. caviae* NCWO: fosmidomycin-resistant mutant derived from NCW.

NT: Not tested.

adenosine 5′-monophosphate. Fosfocytocin was determined to have the structure of cytidylated fosfoxacin.

In the time course of fermentation using strain PK-5, fosfadecin was detected from the early stage to the end of fermentation, while fosfomycin was produced only in the later stage. Fosfadecin seems to be an intermediate in fosfomycin biosynthesis. However, only two of the 12 strains which we isolated as fosfomycin producers, produced fosfadecin, and we have not yet succeeded in converting a strain which produces only fosfomycin into a fosfadecin producing strain. Therefore, fosfadecin may not be a common intermediate in fosfomycin biosynthesis but may be a shunt product. This remains to be solved.

Table 6. Therapeutic efficacy of fosfadecin against experimental bacterial infections in mice.

Organism	Administration route	ED_{50} (mg/kg)
Escherichia coli O-111	sc	259
	po	>800
Staphylococcus aureus 308A-1	sc	449
	po	566

Mice were infected intraperitoneally with 0.5 ml of a suspension of bacteria (10^8 cfu/ml). Groups of five mice at each dose level were given 0.2 ml of an antibiotic solution immediately after infection.

The ED_{50} was calculated from the survival rate at 5 days after infection.

These nucleotidyl compounds showed decreased antibacterial activity. Although nucleotidylation makes the MW of the compound only about three times as high, the antibacterial activity is drastically decreased. This points to the possibility of fosfadecin and fosfocytocin's being inactivated compounds of fosfomycin and fosfoxacin.

In clinical isolates which are resistant to aminoglycoside antibiotics such as streptomycin[4], kanamycin[5] and gentamicin[6], it is well known that the inactivation of these antibiotics by nucleotidylation is one of

the mechanisms of drug resistance. It is possible that the nucleotide antibiotics, fosfadecin and fosfocytocin, which have decreased antibacterial activity, are the inactivated products of fosfomycin and fosfoxacin which are inactivated by the producing bacteria for detoxication. On the other hand, nucleotides act physiologically not only as building blocks for nucleic acid synthesis but also as metabolite carriers, energy donors, second messengers, and cofactors for various enzymes. Therefore, it will be interesting to determine the physiological role of these nucleotide antibiotics.

Experimental

Hydrolysis of Fosfadecin (1)

A solution of the disodium salt of fosfadecin (500 mg) in 1 N NaOH (50 ml) was stirred for 10 hours at 50°C and was then allowed to stand for 15 hours at room temperature. The reaction mixture was adjusted to pH 7 and subjected to activated carbon chromatography (60 ml) with an elution of 8% isobutyl alcohol - 0.02 M ammonia. The eluate was chromatographed on Dowex 50WX2 (H^+ type, 50~100 mesh, 20 ml) eluting with 0.1 M formic acid. The eluate was evaporated to dryness, and the residue was crystallized from water - acetone to give adenosine 5'-monophosphate (190 mg). MP 188~191°C (dec), $[\alpha]_D$ −50.0° (*c* 0.51, 2% NaOH).

Anal Calcd for $C_{10}H_{14}N_5O_7P \cdot H_2O$: C 32.89, H 4.42, N 19.17, P 8.48.
Found: C 33.25, H 4.41, N 19.04, P 8.93.

The other spectral data were identical to those of the authentic sample.

The effluent in the process of the activated carbon chromatography was applied to a column of alumina (60 ml) and eluted with 1 M ammonia water. The eluate was concentrated and applied to a column of Dowex 50WX2 (Na^+ type, 50~100 mesh, 15 ml). The concentrate of the effluent was chromatographed on Bio-Gel P-2 (100~200 mesh, 200 ml) eluting with water. The pure fractions were concentrated and freeze-dried to give a powder of fosfomycin as the disodium salt (126 mg). $[\alpha]_D$ −3.1° (*c* 0.61, H_2O).

Anal Calcd for $C_3H_5O_4PNa_2 \cdot 1\frac{1}{2}H_2O$: C 17.24, H 3.86, P 14.82.
Found: C 17.56, H 3.98, P 14.54.

The other spectral data were identical to those of the authentic sample.

Hydrolysis of Fosfocytocin (2)

To the solution of the disodium salt of fosfocytocin (500 mg) in 0.05 M Tris-HCl buffer (pH 7.4, 300 ml), $MgCl_2$ (258 mg) and nucleotide pyrophosphatase (150 U, snake venom, Sigma) were added and the mixture was incubated at 37°C for 100 minutes. The reaction mixture (300 ml) was chromatographed on a column of activated carbon (30 ml) using 70% acetonitrile - 0.02 M ammonia as the eluent. The eluate was chromatographed on Dowex 50WX2 (H^+ type, 50~100 mesh, 20 ml) eluting with 0.1 M formic acid. The eluate was evaporated to dryness, and the residue was crystallized from water - acetone to give cytidine 5'-monophosphate (217 mg). The physico-chemical data were identical to those of the authentic sample.

The effluent in the process of the activated carbon chromatography was applied to a column of QAE-Sephadex A-25 (Cl^- type, 100 ml) and eluted with 0.02 and 0.05 M NaCl. The active fractions were concentrated, applied to a column of Bio-Gel P-2 (100~200 mesh, 500 ml) and eluted with water. The active fractions were concentrated and again chromatographed on Bio-Gel P-2 (100~200 mesh, 500 ml) eluting with water. The pure fractions were concentrated and freeze-dried to give a white powder of fosfoxacin as the monosodium salt (76 mg).

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References

1) HENDLIN, D.; E. O. STAPLEY, M. JACKSON, H. WALLICK, A. K. MILLER, F. J. WOLF, T. W. MILLER, L. CHAILET, F. M. KAHAN, E. L. FOLTZ, H. B. WOODRUFF, J. M. MATA, S. HERNANDEZ & S. MOCHALES: Phosphonomycin,

a new antibiotic produced by strains of *Streptomyces*. Science 166: 122~123, 1969

2) OKUHARA, M.; Y. KURODA, T. GOTO, M. OKAMOTO, H. TERANO, M. KOHSAKA, H. AOKI & H. IMANAKA: Studies on new phosphonic acid antibiotics. III. Isolation and characterization of FR-31564, FR-32863 and FR-33289. J. Antibiotics 33: 24~28, 1980
3) SHOJI, J.; T. KATO, H. HINOO, T. HATTORI, K. HIROOKA, K. MATSUMOTO, T. TANIMOTO & E. KONDO: Production of fosfomycin (phosphonomycin) by *Pseudomonas syringae*. J. Antibiotics 39: 1011~1012, 1986
4) UMEZAWA, H.; S. TAKASAWA, M. OKANISHI & R. UTAHARA: Adenylstreptomycin, a product of streptomycin inactivated by *E. coli* carring R factor. J. Antibiotics 21: 81~82, 1968
5) NAGANAWA, H.; M. YAGISAWA, S. KONDO, T. TAKEUCHI & H. UMEZAWA: The structure determination of an enzymatic inactivation product of 3',4'-dideoxykanamycin B. J. Antibiotics 24: 913~914, 1971
6) BENVENISTE, R. & J. DAVIES: R-factor mediated gentamicin resistance: A new enzyme which modifies aminoglycoside antibiotics. FEBS Lett. 14: 293~296, 1971