ANKINOMYCIN, A POTENT ANTITUMOR ANTIBIOTIC

Sir:

Ankinomycin, a new member of the pluramycin antibiotics, which is shown here to be deangolosaminylhedamycin, has been isolated together with hedamycin[1] from the culture broth of *Streptomyces* sp. SF2587 (FERM-P 9831). The strain was isolated from a soil sample collected in Takatsuki-city, Osaka Prefecture, Japan. The antibiotic is active against Gram-positive bacteria, and exhibits potent antitumor activity against P388 leukemia in mice. These activities are superior to those of hedamycin. In this communication, the isolation, characterization and structural elucidation of the antibiotic are reported.

Fermentation of *Streptomyces* sp. SF2587 was carried out at 28°C for 113 hours in a 300-liter fermentor with an agitation rate of 200 rpm and an airflow of 100 liters/minute. The medium (200 liters) consisted of maltose syrup 2.4%, soybean meal 1.2%, cotton seed meal 0.6%, soluble vegetable protein 0.3%, soybean oil 0.18%, $CaCO_3$ 0.12%, $MgSO_4 \cdot 7H_2O$ 0.1%, $NaB_4O_7 \cdot 7H_2O$ 0.1% and $ZnSO_4 \cdot 7H_2O$ 0.001% (pH 7.0). Antibiotic activity was assayed by the paper-disc method using *Micrococcus luteus* as the test organism, and by measurements of the cytotoxicity on P388 cells *in vitro*.

The fermentation broth (300 liters, pH 7.8) from two fermentors was adjusted to pH 2.5 with dilute HCl and then filtered with the aid of diatomaceous earth. Hedamycin was found in the filtrate as a major product. Ankinomycin

Table 1. ^1H NMR data of ankinomycin and hedamycin.

Proton	Ankinomycin		Hedamycin
	$CDCl_3$	C_5D_5N	$CDCl_3$
3-H	6.45 s	6.68 s	6.47 s
6-H	8.04 s*	8.14 s	8.01 s*
8-H	7.81 d (8.2)	8.03 d (8.2)	
9-H	7.86 d (8.2)	8.13 d (8.2)	8.33 s*
11-OH	13.42 s		14.03 s*
13-H_3	2.98 s*	3.01 s	2.99 s
15-H_3	1.96 s	2.10 s	1.96 s
16-H	3.35 d (4.7)	3.70 d (5.7)	3.34 d (4.9)
17-H	2.90 dd (2.3, 4.7)	3.16 dd (2.2, 5.7)	2.90 dd (2.3, 4.9)
18-H	3.12 dq (2.3, 5.5)	3.26 dq (2.2, 5.1)	3.12 dq (2.3, 5.1)
19-H_3	1.45 d (5.5)	1.31 d (5.1)	1.45 d (5.1)
2′-H	4.09 dq (2.0, 6.6)	4.37 dq (3.6, 6.6)	4.06 dq (1.3, 6.4)
3′-H	3.45 d* (2.0)	3.83 d* (3.6)	3.37 d*
4′-$N(CH_3)_2$	2.27 s*	2.38 s	2.24 s
5′-H_2	2.18 dd (5.8, 14.1)	1.88 dd (7.7, 13.7)	2.28 dd (6.4, 13.8)
	2.33 dd (6.3, 14.1)	2.57 dd (5.1, 13.7)	2.55 dd (3.6, 13.8)
6′-H	5.47 dd (5.8, 6.3)	5.79 dd (5.1, 7.7)	5.45 dd (3.6, 6.4)
7′-H_3	1.47 d (6.6)	1.76 d (6.6)	1.50 d (6.4)
8′-H_3	0.83 s*	1.04 s*	0.73 s
2″-H			3.57 dq (5.9, 8.7)
3″-H			3.23 dd (8.7, 10.0)
4″-H			2.93 ddd (3.8, 10.0, 12.1)
4″-$N(CH_3)_2$			2.36 s
5″-H_2			1.34 ddd (10.0, 12.1, 13.0)
			2.26 m
6″-H			5.46 dd (1.3, 10.0)
7″-H_3			1.43 d (5.9)

* Broad signal.

Measured at 400 MHz; chemical shifts in ppm from TMS, J in Hz.

in the mycelial cake was extracted with 70% aq acetone (110 liters, 15 μg/ml). After the extract was adjusted to pH 7.5 with 10 N NaOH, acetone was removed by evaporation under reduced pressure. The antibiotic in the aq soln was extracted with EtOAc (30 liters). The extract was concentrated to dryness under reduced pressure. The residue was washed with *n*-hexane and dried to give a dark-red residue (13.9 g). The residue was chromatographed on a silica gel column developed with $CHCl_3$ and mixtures of $CHCl_3$ and MeOH (100:1, 50:1, 20:1, 10:1 and 5:1) to give a yellowish powder (6.4 g). The powder was further purified by chromatography on a silica gel column in a similar method as described above to give an yellow powder of pure ankinomycin (281 mg): MP 234°C (dec); $[\alpha]_D^{28}$ +233° (*c* 0.1, $CHCl_3$); high resolution (HR)-MS *m/z* 589.2266, M^+, calcd for $C_{33}H_{35}NO_9$: 589.2309; UV λ_{max}^{MeOH} nm (ε) 243 (65,400), 267 (sh, 39,460), 422 (13,310); $\lambda_{max}^{0.1N\ HCl-MeOH}$ nm (ε) 243 (73,150), 267 (sh, 44,180), 421 (15,260); $\lambda_{max}^{0.1N\ NaOH-MeOH}$ nm (ε) 255 (65,440), 285 (sh, 21,790), 337 (15,730), 544 (10,250); IR ν (KBr) cm^{-1} 3430 (OH), 1655 (CO), 1595 (C=C). ^1H and ^{13}C NMR spectra of ankinomycin are shown in Tables 1 and 2. Ankinomycin was soluble in $CHCl_3$ and acidic water, hardly soluble in MeOH, EtOH, EtOAc and acetone, and insoluble in water. Silica gel TLC (E. Merck, Art 5714) developed with $CHCl_3$-MeOH (5:1) and BuOH - AcOH - H_2O (4:1:2) showed the Rf values 0.23 and 0.37, respectively.

The UV spectrum showed close similarity to that of pluramycin A[2,3] and related antibiotics[1,4,5] having the indomycinone[6~9] chromophore (11-hydroxy-4*H*-anthraceno[1,2-b]pyran-4,7,12-trione system). The ^1H and ^{13}C NMR spectra were closely related to those of hedamycin with the exception of the signals due to the aminosugar (angolosamine)[10] moiety at C-8 in hedamycin[7,11] (Tables 1 and 2).

The HR-MS (*m/z* 589.2266, M^+) suggested that the aminosugar (angolosamine, $C_8H_{16}NO_2$) moiety at C-8 in hedamycin is replaced by a hydrogen, which was observed as a characteristic AB type spin system at 8-H and 9-H in the ^1H NMR spectrum of ankinomycin. On comparison of the ^1H and ^{13}C NMR spectra with those of hedamycin, the values of chemical shifts and coupling constants of an aminosugar (*N,N*-dimethylvancosamine)[10] at C-10 and the bi-

Table 2. ^{13}C NMR data of ankinomycin and hedamycin.

Carbon	Ankinomycin	Hedamycin
C-2	166.3 s	166.6 s
C-3	110.0 d	110.0 d
C-4	178.8 s	179.3 s
C-4a	126.5 s	126.0 s
C-5	149.9 s	149.9 s
C-6	126.0 d	126.1 d
C-6a	136.3 s	137.5 s
C-7	181.5 s	183.3 s
C-7a	130.7 s	126.2 s
C-8	119.3 d	139.8 s
C-9	133.2 d	133.0 d
C-10	140.7 s	138.5 s
C-11	159.5 s	159.5 s
C-11a	116.1 s	116.2 s
C-12	187.7 s	188.0 s
C-12a	119.8 s	119.4 s
C-12b	156.2 s	156.1 s
C-13	24.1 q	24.2 q
C-14	57.7 s	57.8 s
C-15	14.4 q	14.5 q
C-16	63.9 d	64.0 d
C-17	55.4 d	55.6 d
C-18	51.8 d	52.1 d
C-19	17.2 q	17.2 q
C-2′	67.9 d	67.1 d
C-3′	71.0 d	70.9 d
C-4′	57.7 s	57.8 s
4′-$N(CH_3)_2$	37.1 q	36.8 q
C-5′	35.0 t	33.5 t
C-6′	68.2 d	69.8 d
C-7′	17.5 q	17.6 q
C-8′	13.2 q	12.3 q
C-2″		77.4 d
C-3″		72.0 d
C-4″		67.2 d
4″-$N(CH_3)_2$		40.4 q
C-5″		28.6 t
C-6″		75.2 d
C-7″		18.9 q

Measured at 100 MHz in $CDCl_3$; chemical shifts in ppm from TMS.

oxirane side chain at C-2 in ankinomycin were nearly identical with those of the corresponding resonances of hedamycin[7,11]. In addition to these results, nuclear Overhauser effects (NOE's) were observed with 3′-H and 6′-H upon irradiation of 7′-H, and 2′-H and 3′-H upon irradiation of 8′-H in the ^1H-^1H NOE difference spectrum. These results indicated that the conformation of the aminosugar moiety of ankinomycin in

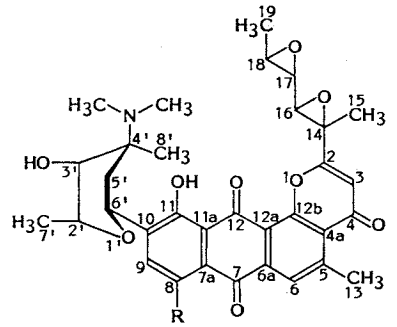
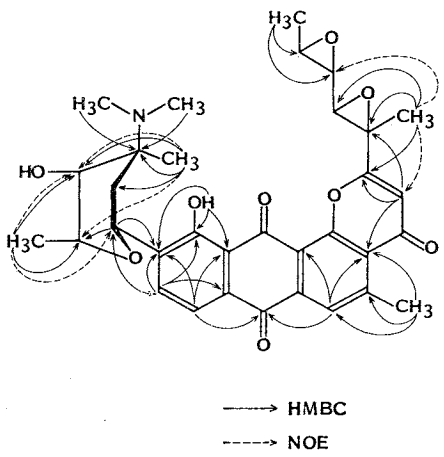


Fig. 1. HMBC and NOE experiments of ankinomycin.



$CDCl_3$ soln should be a boat form as in the case of kidamycin[10] and hedamycin[11] rather than the chair form reported in isokidamycin[10] and chromoxymycin[12].

The configuration of the bioxirane side chain (C-14 to C-19) of ankinomycin should be trans-substituted with an '*O*-cis'[13,14] conformation as in hedamycin in view of the closely similar chemical shifts and coupling constants (J_{C-H} 178.2, 176.9 and 172.1 Hz at C-16, C-17 and C-18; $J_{H\text{-}17/H\text{-}18}$ 2.3 Hz). Observation of an NOE with 17-H upon irradiation of 15-H supported these results. On the other hand, NOE's with 3-H upon irradiation of 15-H in both ankinomycin and hedamycin were observed. This evidence indicates that the methyl group

Table 3. Antimicrobial activity of ankinomycin.

Organism	MIC (μg/ml)
Staphylococcus aureus 209P JC-1	0.39
S. aureus Smith S-424	0.39
S. aureus No. 26	0.39
S. epidermidis ATCC 14990	1.56
S. epidermidis 109	1.56
Enterococcus faecalis ATCC 8043	0.78
Bacillus anthracis No. 119	0.78
Escherichia coli NIHJ JC-2	>100
E. coli No. 29	>100
E. coli W 3630 RGN 823	>100
E. coli JR66/W677	100
Citrobacter freundii GN346	25
Salmonella typhi O-901-W	25
S. enteritidis No. 11	>100
S. typhimurium LT-2	>100
Shigella sonnei EW 33 Type 1	25
Klebsiella pneumoniae PCI 602	>100
K. pneumoniae 22 #3038	>100
Proteus vulgaris OX19	12.5
P. mirabilis GN310	100
Morganella morganii Kono	>100
Serratia marcescens MB-3848	>100
Pseudomonas aeruginosa MB-3829	50
Xanthomonas maltophilia M-0627	>100

Determined on a Sensitivity Disk Agar-N medium (Nissui Seiyaku).

(15-H) on the bioxirane side chain of both antibiotics is close to 3-H in $CDCl_3$ soln[3,15].

The position of attachment of the aminosugar to the chromophore of ankinomycin through a *C*-glycosyl linkage was determined by observation of the long range couplings of 6'-H to C-9 and C-10, and 9-H to C-6'. The point of attachment of the bioxirane side chain to the chromophore was confirmed by observation of the long range couplings of 3-H to C-2 and C-14, and 15-H to C-2, C-14 and C-16 in heteronuclear multiple bond correlation spectrum (HMBC) experiments. Assignments of all the protons and carbons of ankinomycin were established by the ^1H-^1H selective decoupling, ^1H-^{13}C shift correlation spectroscopy (^1H-^{13}C COSY) and HMBC experiments. The results of NOE and HMBC experiments are summarized in Fig. 1. From the above spectral analyses, the structure of ankinomycin was determined.

Ankinomycin is strongly active against Gram-positive bacteria and weakly against Gram-negative bacteria, as shown in Table 3. Marked increases in life span (ILS) were observed in

Table 4. Antitumor activities of ankinomycin and hedamycin against P388 leukemia in mice.

Ankinomycin			Hedamycin	
Dose (mg/kg)	ILS (%)		Dose (mg/kg)	ILS (%)
	P388	P388/ADR[a]		P388
1.0	Toxic		10	Toxic
0.5	126	58	5	55
0.25	67	44	2.5	44
0.125	48	35	1.25	34
0.063	33		0.63	13

[a] Multiple drug-resistant subline of P388 leukemia.

experiments with a single ip treatment of ankinomycin against mice ip-implanted P388 and multiple drug-resistant leukemia cells (P388/ADR). The antitumor activity of ankinomycin was higher than that of hedamycin (Table 4). The ip LD_{50} value of ankinomycin was 0.5~1.0 mg/kg in mice.

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