KANAGAWAMICIN, A NEW AMINONUCLEOSIDE ANALOG ANTIBIOTIC FROM ACTINOPLANES KANAGAWAENSIS

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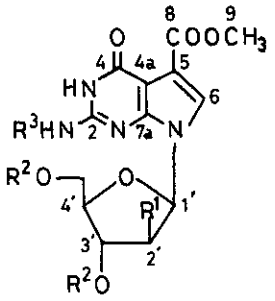
Abstract —— A new aminonucleoside, kanagawamicin (Ia), is produced by a strain belonging to Actinoplanes kanagawaensis. The structure of Ia has been deduced from physico-chemical data obtained using the natural compound and its acetates (Ib, Ic and Id).

During the course of our screening program for antibiotics, a new aminonucleoside, named kanagawamicin, was isolated from the fermentation broth of a strain belonging to Actinoplanes kanagawaensis. The antibiotic showed antitumor activity and weak antibacterial activity against Gram-negative bacteria. This paper deals with the structure of kanagawamicin (Ia).

The fermentation broth (100 liters) was purified by successive column chromatography on Diaion HP-20 (eluted with 10% acetone), Amberlite IRC-50 (H^+ form) and Sephadex LH 20 (eluted with 90% MeOH) to give Ia (0.1 g) as a colorless amorphous powder, $C_{13}H_{17}N_5O_6 \cdot H_2O$,[1] mp 225-258 °C(dec), $[\alpha]_D^{25}$ -14.0° (c=1, DMSO), $\nu_{OH,\ NH}^{KBr}$ 3200-3400 cm^{-1}, $\nu_{C=O}^{KBr}$ 1710 and 1630 cm^{-1}.

For the purpose of further spectral analysis, the following acetates of Ia were prepared. Acetylation of Ia with Ac_2O-MeOH at 0 °C gave an N-monoacetate (Ib), $C_{15}H_{19}N_5O_7$, mp 205-207 °C, in 99% yield. Treatment of Ia with Ac_2O-pyridine at room temperature afforded an N,O,O-triacetate (Ic), $C_{19}H_{23}N_5O_9$, mp 170-171 °C, and an N,N,O,O-tetraacetate (Id), $C_{21}H_{25}N_5O_{10}$, amorphous powder, in 20% and 10% yields, respectively. The ^{1}H-NMR (PMR) and mass spectral data of Ia-d are shown in Tables I and II. Comparison of ^{13}C-NMR (CMR) data of Ia and Ic with those of cadeguomycin (II)[2] and 2'-amino-2'-deoxyguanosine (IIIa)[3] are compiled in Table III.

These spectral data shown in Tables I-III suggested that Ia is an aminonucleoside being an analog of cadeguomycin (II).[2] The mass spectra of Ia-c exhibited a fragment ion peak at m/z 208 (aglycon + 1) indicating that the aglycon moiety of Ia was $C_8H_7N_4O_3$ (MW 207) having $COOCH_3$, NH_2 and NH (or OH) groups which were consistent with their PMR data. The UV spectral data of Ia

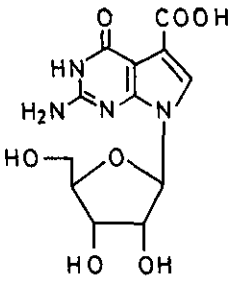


Ia R^1=NH_2, R^2=R^3=H

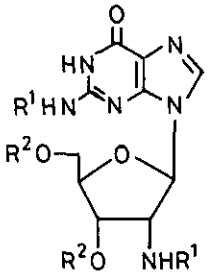
Ib R^1=$NHCOCH_3$, R^2=R^3=H

Ic R^1=$NHCOCH_3$, R^2=$COCH_3$, R^3=H

Id R^1=$NHCOCH_3$, R^2=R^3=$COCH_3$



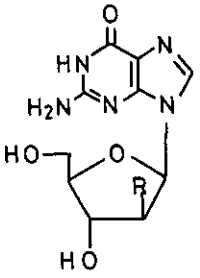
II



IIIa R^1=R^2=H

IIIb R^1=$COCH_3$, R^2=H

IIIc R^1=R^2=$COCH_3$



IVa R=NH_2

IVb R=N_3

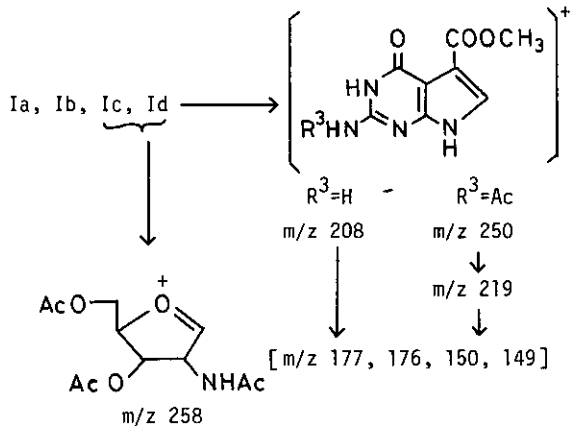
Table I ^{1}H-NMR (PMR) Data of Kanagawamicin (Ia) and Its Acetates (Ib, Ic and Id)

	position	Ia[a]	Ib[a), f]	Ic[a), g]	Id[b), h]
Chemical Shifts (δ)	6-H	7.70 (s)[c]	7.60 (s)	7.56 (s)	7.56 (s)
	5-$COOCH_3$	3.69 (s)	3.70 (s)	3.72 (s)	3.85 (s)
	2-NH_2	6.55 (bs→0)[d]	6.35 (bs→0)	6.37 (bs→0)	-
	3-NH	na[e]	10.40 (bs→0)	10.54 (bs→0)	9.50 (bs→0)
	1'-H	6.17 (d)	6.24 (d)	6.33 (d)	6.26 (d)
	2'-H	3.3-4.1 (m)	4.42 (dt→t)	4.79 (m→dt)	4.99 (m→dd)
	3'-H		4.18 (m→t)	5.38 (t→t)	5.98 (t→t)
	4'-H		3.5-3.9 (m)	4.18 (m→m)	4.80 (m→m)
	5'-H_2			4.30 (m→m)	4.22 (m→m)
	2'-$NHCOCH_3$	-	7.87 (d→0)	8.16 (d→0)	6.30 (bs→0)
	2'-$NHCOCH_3$	-	1.60 (s)	1.60 (s)	1.73 (s)
Coupling Constants (Hz)	$J_{1',2'}$	6.0	6.0	6.5	7.2
	$J_{2',3'}$	na	6.1	7.2	8.4
	$J_{3',4'}$	na	6.1	7.2	8.4

a) 100 MHz in DMSO-d_6. b) 100 MHz in $CDCl_3$. c) Splitting. d) →: Change of splitting on deuteration with D_2O. 0: Disappearance of signal on deuteration. e) Not assigned. f) Other data; 3'-OH δ 5.63 (d→0), 5'-OH δ 5.20 (bs→0), $J_{2'NH,2'}$=8.0 Hz, $J_{3'OH,3'}$=5.0 Hz. g) Other data; $OCOCH_3$ δ 2.09 (s, 6H), $J_{2'NH,2'}$=7.8 Hz. h) Other data; $OCOCH_3$ δ 2.10 (s, 3H) and δ 2.18 (s, 3H), 2-$NHCOCH_3$ δ 2.28 (s, 3H)

Table II Mass Spectral Data of Kanagawamicin (Ia) and Its Acetates (Ib, Ic and Id)

Fragmentation Pattern



m/z	Relative Intensity (%) Ia[a]	Ia[b]	Ib[a]	Ic[a]	Id[a]
149	100	-	100	6	21
150	68	-	43	25	16
176	14	-	36	28	56
177	15	-	48	35	52
208	8	5	80	100	100
219	-	-	-	-	35
250	-	-	-	-	64
258	-	-	-	6	15
M^+ (m/z)	- (339)	35 (339)	10 (381)	10 (465)	18 (507)

a) EI-MS. b) FD-MS, 100%=m/z 340 (M^++1)

Table III Comparison of ^{13}C-NMR (CMR) Chemical Shifts (δ) in Kanagawamicin (Ia) and Its Triacetate (Ic), Cadeguomycin (II) and 2'-Amino-2'-deoxyguanosine (IIIa)

position	Ia[a]	II[b]	Ic[a], d)	IIIa[c]
C-2	153.46 s[e]	153.32 s	153.13 s	154.93 s
C-4	157.57 s	161.39 s	156.95 s	160.42 s
C-4a	97.30 s	96.31 s	96.99 s	117.85 s
C-5	109.12 s	110.96 s	110.12 s	-
C-6	126.43 d	125.64 d	125.63 d	139.11 d
C-7a	151.96 s	152.18 s	152.17 s	152.51 s
C-8	163.37 s	162.67 s	163.10 s	-
C-1'	84.49 d	86.17 d	81.54 d	87.56 d
C-2'	60.30 d	74.14 d	55.10 d	58.15 d
C-3'	75.59 d	70.47 d	73.72 d	72.81 d
C-4'	84.19 d	85.27 d	77.30 d	89.21 d
C-5'	60.42 t	61.29 t	62.58 t	63.00 t
C-9	50.75 q	-	50.77 q	-

a) 20 MHz in DMSO-d_6. b) These data were cited from reference 2). (100 MHz in DMSO-d_6).
c) These data were cited from reference 3). (25 MHz in D_2O). d) Other carbons: $CO\underline{C}H_3$; δ 21.85[q], 20.49[q] and 20.32[q]. $\underline{C}OCH_3$; δ 169.96[s], 169.87[s] and 169.17[s]. e) Splitting.

[$\lambda_{max}^{H_2O}$ 233 nm (log ε 4.20), 272 (3.80), 298 (3.84); $\lambda_{max}^{0.1N\ HCl}$ 231 nm (log ε 4.15), 272 (3.80), 297 (3.84)] showed a closed similarity to those of II [$\lambda_{max}^{H_2O} = \lambda_{max}^{0.1N\ HCl}$ 232 nm (log ε 4.30), 272 (3.84), 298 (3.88)].[2] As shown in Table III, the CMR signals due to the aglycon moiety of Ia and Ic appeared at comparable position with those of II[2, 4] except a signal at δ 50.75-50.77 attributable to the methyl carbon (C-9, $COO\underline{C}H_3$) of the ester group. The PMR signal at δ 7.70 (DMSO-d_6) of Ia (Table I) was assigned as 6-H by comparison with the signal at δ 7.78 (DMSO-d_6) due to 6-H of II.[2] These results suggested that the aglycon moiety of Ia is methyl 2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidine-5-carboxylate. The spectral data of Ia (the fragment ion peak at m/z 208 described above in its mass spectrum, a signal at δ 6.17 attributable to 1'-H in its PMR spectrum, and a signal at δ 84.49 due to C-1' in its CMR spectrum) indicated that Ia has an N-glycosyl bond.[2, 3] As in the case of the known N-7-glycosides of 7H-pyrrolo[2,3-d]-pyrimidine derivatives, such as the modified nucleoside Q[5] and II[2], the aglycon of Ia was not obtained by acidic hydrolysis.[2, 6]

The mass spectra of Ic and Id exhibiting a fragment ion peak at m/z 258 depicted in Table II indicated that the sugar moiety of Ia is an amino-deoxyfuranose[7] which was substantiated by their PMR data. By comparison of CMR data shown in Table III, it was noted that the chemical shift of one carbon (δ 60.30) of the sugar moiety of Ia was similar to that of C-2' (δ 58.15) of IIIa, but higher than those of C-2' (δ 74.14) and C-3' (δ 70.47) of II. These facts suggested that the amino group of Ia is attached to C-2' or C-3' position of the furanose moiety. Assignment of the position of the amino group in Ia as C-2' position was accomplished by the PMR spectral analysis of 2'-N-acetates, Ib and Ic, as shown in Table I. All signals due to 1'-H, 2'-H, 3'-H and 2'-NHAc of these acetates were fully assigned by means of spin decoupling and deuteration with D_2O. Comparison of the PMR data shown in Table I with those of the reported nucleosides having 2-amino-2-deoxyfuranose moiety provided further information on the configuration of the aminosugar moiety of Ia. As already noted,[3] the chemical shift of the anomeric proton (1'-H) being cis-configuration to 2'-H appeared at lower field (usually at about 0.5 ppm) than that of 1'-H being trans-configuration to 2'-H in the PMR spectra. The chemical shift of 1'-H of Ia (δ 6.17, d, J=6.0 Hz, DMSO-d_6) was similar to those of 9-(2-amino-2-deoxy-β-D-arabinofuranosyl)guanine (IVa) (δ 5.98, d, J=6.0 Hz, DMSO-d_6)[8] and 9-(2-amino-2-deoxy-β-D-arabinofuranosyl)adenine (δ 6.22, d, J=6.5 Hz, DMSO-d_6)[8], but different from that of IIIa [9-(2-amino-2-deoxy-β-D-ribofuranosyl)guanine] (δ 5.44, d, J=6.0 Hz, DMSO-d_6).[9] The chemical shift of 1'-H of Ia was 0.73 ppm lower than that of IIIa in DMSO-d_6. Thus, it was suggested that Ia has a 1'-H-2'-H-cis-configuration. As shown in Table I, the first order coupling constant of $J_{1',2'}$, $J_{2',3'}$ and $J_{3',4'}$-values of Ia and its acetates (Ib-d) were 6.0-7.2 Hz, 6.1-8.4 Hz

and 6.1-8.4 Hz, respectively. And in the PMR spectra of Ib-c, the signal due to 3'-H observed as triplet after deuteration with D_2O indicating that $J_{2',3'}$- and $J_{3',4'}$-values of them resembled each other. The $J_{2',3'}$- and $J_{3',4'}$-values of Ib-d were closely similar to those of 9-(2-azido-2-deoxy-β-D-arabinofuranosyl)guanine (IVb) ($J_{2',3'}$=8.0 Hz, $J_{3',4'}$=7.0 Hz, DMSO-d_6)[8], 9-(2-azido-2-deoxy-β-D-arabinofuranosyl)adenine ($J_{2',3'}$=$J_{3',4'}$=8.0 Hz, DMSO-d_6)[8] and 1-(2-deoxy-3,5-di-O-*p*-nitrobenzoyl-2-trifluoroacetamido-α-D-arabinofuranosyl)-4-methoxy-2(1*H*)-pyrimidinone [$J_{2',3'}$=$J_{3',4'}$=6.5 Hz (pyridine-d_5 or acetone-d_6); $J_{2',3'}$=6.8 Hz, $J_{3',4'}$=6.5 Hz ($CDCl_3$)][10], but different from those of IIIb ($J_{2',3'}$=5.5 Hz, $J_{3',4'}$=2 Hz, D_2O)[3] and IIIc ($J_{2',3'}$=5.8 Hz, $J_{3',4'}$=2 Hz, $CDCl_3$-CD_3OD).[3] These facts indicated that the aminosugar moiety of Ia is 2-amino-2-deoxyarabinose.

The remaining problem is the mode of the glycosylic linkage of Ia. In the PMR spectrum of the N-monoacetete (Ib), the signal due to 2'-N-acetyl group resonanced at higher field (δ 1.60, DMSO-d_6). One of the acetyl signal of the acetates, Ic and Id, also appeared at higher field, δ 1.60 (DMSO-d_6) and δ 1.73 ($CDCl_3$), respectively (Table I). On the other hand, the chemical shifts of the corresponding 2'-N-acetyl signal of IIIb and IIIc have been reported as δ 2.01 (D_2O) and δ 1.94 ($CDCl_3$-CD_3OD).[3] These evidences were readily reconciled with the β-glycosylic linkage of Ia in which the 2'-N-acetyl group is in the *cis*-configuration to the pyrrolo[2,3-*d*]pyrimidine ring having an anisotropic effect.

Consequently, the structure of Ia was deduced to be methyl 2-amino-3,4-dihydro-4-oxo-7-[2-amino-2-deoxy-β-D (or L)-arabinofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylate[11] except for its absolute configuration. The basic structure of the aglycon moiety of Ia is identical with those of the modified nucleoside Q[5] and cadeguomycin (II)[2], but the occurrence of 2-amino-2-deoxyarabinose as a sugar moiety of natural nucleosides was found for the first time.

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11) Although Ia is a methyl ester, the presence of Ia was detected in the fermentation broth and the early process of purification of Ia without use of MeOH. Thus, kanagawamicin (Ia) is not an artifact.

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