STUDIES ON A NEW NUCLEOSIDE ANTIBIOTIC, DAPIRAMICIN

II. ISOLATION, PHYSICO-CHEMICAL AND BIOLOGICAL CHARACTERIZATION

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New nucleoside antibiotics, dapiramicins A and B produced by a *Micromonospora* sp. SF-1917, have been isolated by column chromatography on Diaion HP-20 and silica gel. Physico-chemical properties suggested that they are disaccharide nucleosides. Dapiramicin A underwent epimerization, under acidic condition, into epidapiramicin A. Although dapiramicin A generally exhibits no *in vitro* activity, it is very effective against the sheath blight of rice plants caused by *Rhizoctonia solani* in a green house test.

In a previous paper[1], the producing organism, assay method and fermentation of a new nucleoside antibiotic, dapiramicin, were described. In a subsequent study, a minor component was obtained, in addition to dapiramicin, in the fermentation broth of *Micromonospora* sp. SF-1917. So, the former antibiotic was named dapiramicin A and the latter one dapiramicin B. Dapiramicin A is highly effective in the control of sheath blight, a destructive disease of rice plants, but dapiramicin B is less effective.

This paper is concerned with the isolation, physico-chemical and biological characterization of dapiramicins A and B. The structural study[2] and the details of biological properties[3] will be described elsewhere.

Isolation

The fermented broth after 120 hours was filtered with the aid of diatomaceous earth (Hyflo Supercel, Johns-Manville) and the filtrate (7.7 liters) was passed through a column of Diaion HP-20 (800 ml, Mitsubishi Chemical Industries, Ltd.). The column was washed with 20% aqueous ethanol and eluted with 50% aqueous ethanol. The first effluent (700 ml) was discarded, and the second one (2.0 liters) was collected and concentrated to dryness. The residue was extracted with methanol (50 ml) and evaporation of the methanol extract afforded 8.1 g of crude preparation of dapiramicins (12% purity as dapiramicin A). This crude material (8.0 g) was dissolved in 15 ml of methanol and charged onto a column of silica gel (1,000 ml). The column was developed with a mixture of ethyl acetate and methanol (5: 1) and 16-ml fractions were collected. Dapiramicin A was eluted in fractions Nos. 66~116, and evaporation gave 790 mg of pure antibiotic which was crystallized from aqueous methanol. From fractions Nos. 145~180, dapiramicin B was recovered and crystallized from aqueous methanol to give 71 mg of colorless needles. Through this purification procedure, the antibiotic was monitored by high performance liquid chromatography (HPLC)[1] and thin-layer chromatography (TLC).

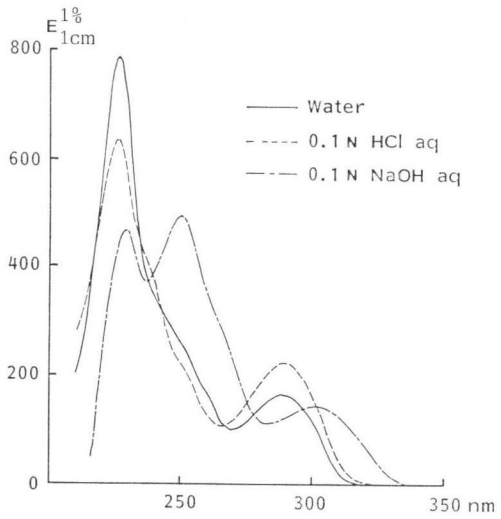
Physico-chemical Properties

Dapiramicins A and B have similar solubilities, *i.e.*, they are soluble in *N*,*N*-dimethylformamide and acetic acid, less soluble in methanol and water, and almost insoluble in acetone, chloroform, ethyl acetate and ethyl ether. Both of them show positive color reactions to Greig-Leaback, potassium permanganate, and ferric cyanide-$FeCl_3$, but were negative to the ninhydrin reagent. Table 1 summarizes the physico-chemical properties of dapiramicins A and B. The elemental and mass analyses established the molecular formula of dapiramicin A as $C_{21}H_{29}N_5O_{10}\cdot H_2O$.

The UV spectra of dapiramicin A are shown in Fig. 1; its IR spectrum is illustrated in Fig. 2, which shows a characteristic absorption band at 2235 cm^{-1} due to a nitrile group in addition to –OH and –NH bands (3405 cm^{-1}).

The 100 MHz ^1H NMR spectrum of dapiramicin A in D_2O - CD_3OD (1: 1) (Fig. 3) shows one aromatic ring proton at 7.44 ppm, two anomeric protons at 4.20 ppm and 5.55 ppm and three methyl signals assigned to one C–CH_3 (1.07 ppm) and two O–CH_3 (3.35, 3.86 ppm) groups. These properties of dapiramicin A suggested that this antibiotic belongs to the class of disaccharide nucleosides. The structure was later determined to be 2-[4′-(4″-*O*-methyl-β-D-glucopyranosyl)-6′-deoxy-α-D-glucopyranosyl]amino-5-cyano-4-methoxy-7*H*-pyrrolo[2,3-d]pyrimidine as shown in Fig. 4 by SETO *et al.*[2].

Fig. 1. UV spectra of dapiramicin A.



On mild acid treatment of dapiramicin A (*i.e.*, in acetic acid, at room temperature for 3 days), mutarotation was observed, and the antibiotic underwent epimerization to give epidapiramicin A. The physico-chemical properties of epidapiramicin A are summarized in Table 1, and it was identified as the β-anomer of dapiramicin A at the glucopyranosylamine bond as shown in Fig. 4[2].

Table 1. Physico-chemical properties of dapiramicins.

	Dapiramicin A	Epidapiramicin A	Dapiramicin B
Appearance	Colorless needles	Colorless needles	Colorless needles
mp	220~222°C	222~224°C	241~243°C
UV $\lambda_{max}^{H_2O}$ ($E_{1cm}^{1\%}$)	227 nm (787), 289 nm (169)	228 nm (930), 292 nm (157)	227 nm (804), 290 nm (138)
IR (KBr, cm^{-1})	3405, 2235, 1623, 1588, 1072	3360, 2235, 1625, 1593, 1540, 1325, 1108	3360, 2215, 1620, 1600, 1545, 1065
$[\alpha]_D^{20}$ (*c* 1.0)	+117° (MeOH)	−14.4° (AcOH)	−37.6° (50% AcOH)
Elemental analysis (%)	C 47.50, H 5.46, N 12.81, O 32.12	C 46.18, H 5.44, N 12.37	C 47.38, H 5.45, N 13.02, O 33.26
MW (FD-MS)	511	511	527
Molecular formula	$C_{21}H_{29}N_5O_{10}\cdot H_2O$	$C_{21}H_{29}N_5O_{10}\cdot H_2O$	$C_{21}H_{29}N_5O_{11}\cdot H_2O$
Silica gel TLC*: Rf	0.68	0.64	0.35

* Solvent system; EtOAc - MeOH (2: 1).

Fig. 2. IR spectrum of dapiramicin A (KBr).

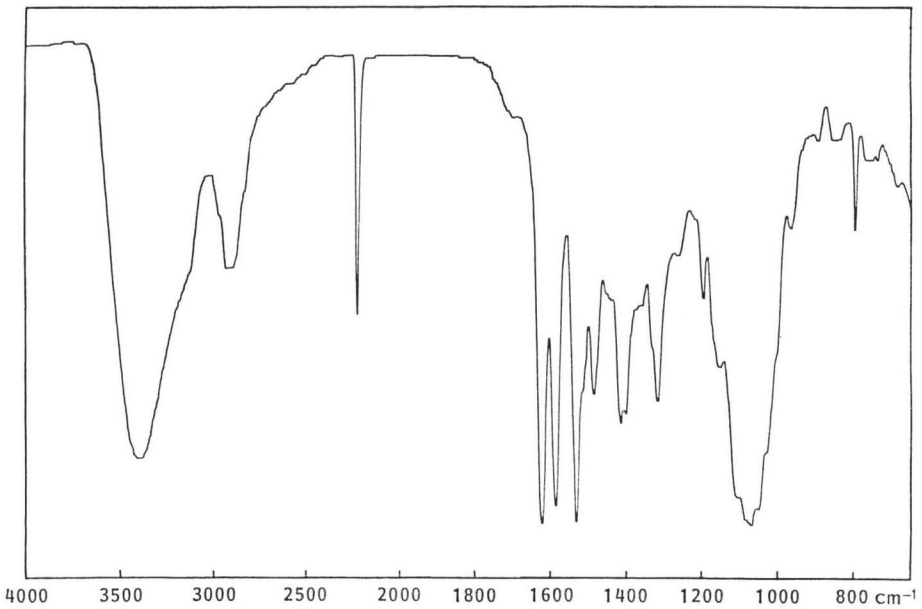


Fig. 3. ^1H NMR spectrum of dapiramicin A (100 MHz, D_2O - CD_3OD, 1: 1).

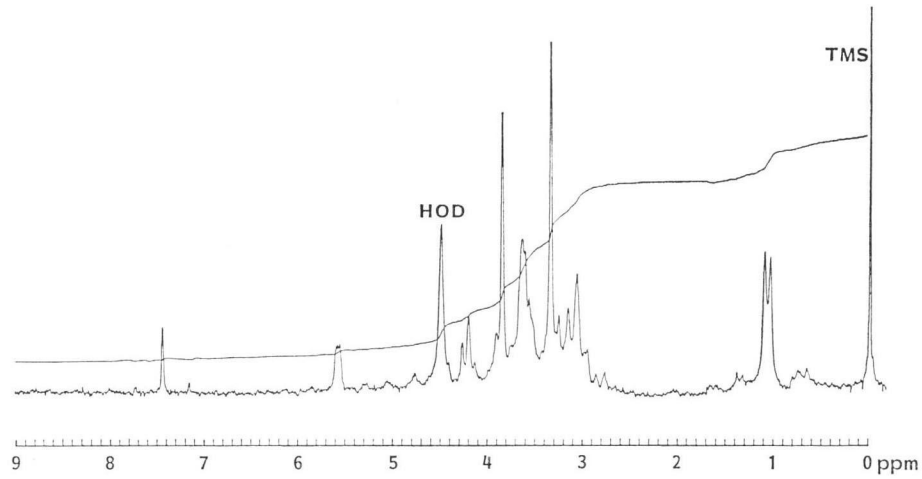
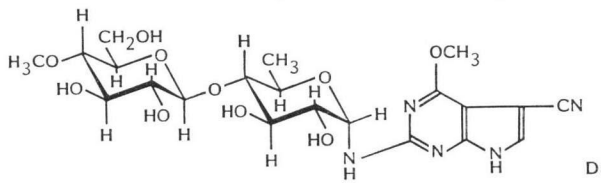
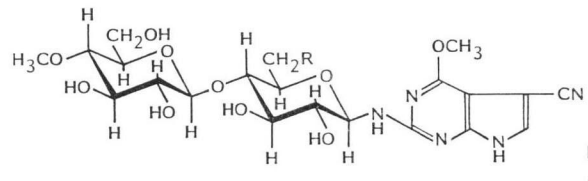


Fig. 4. Structures of dapiramicins.



Dapiramicin A



Epidapiramicin A R = H

Dapiramicin B R = OH

Fig. 5. IR spectrum of dapiramicin B (KBr).

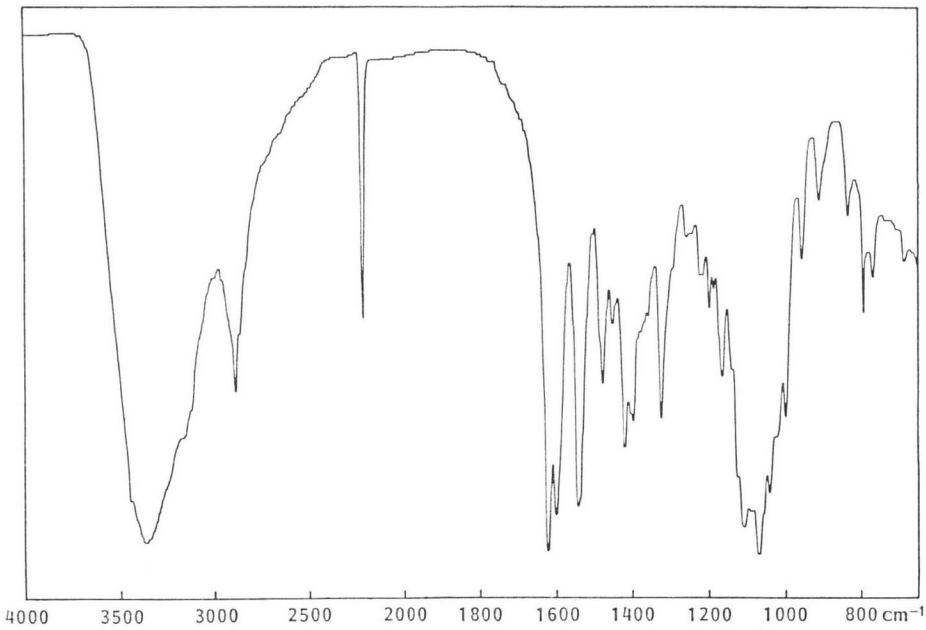
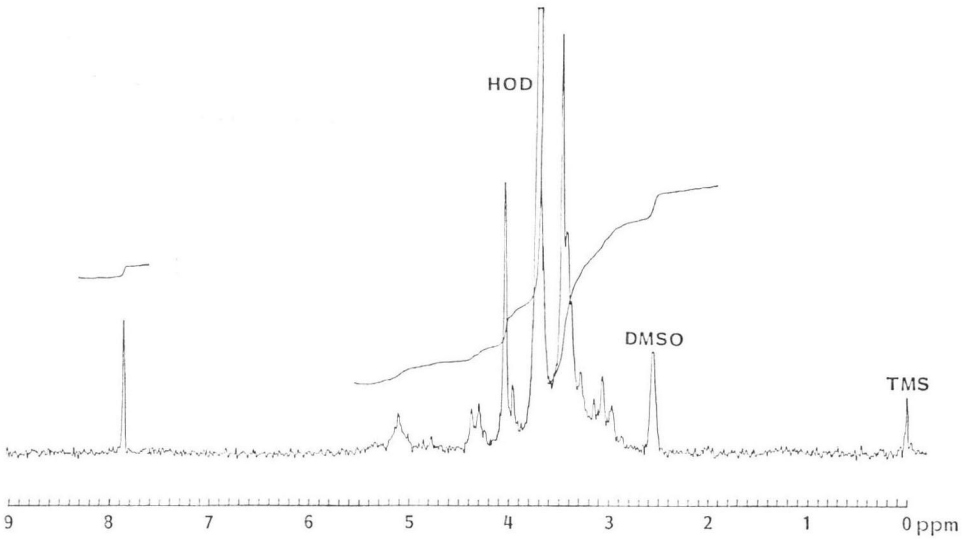


Fig. 6. ^{1}H NMR spectrum of dapiramicin B (100 MHz, DMSO-d_6 - D_2O, 10: 1).



Dapiramicin B and dapiramicin A are closely related, as evidenced by their similar UV (Table 1) and IR spectra (Fig. 5), except for reversed optical rotation sign (Table 1). Dapiramicin B contains one oxygen atom more than dapiramicin A, and *C*-methyl signal (1.07 ppm) observed in dapiramicin A disappeared in the ^{1}H NMR spectrum of dapiramicin B (Fig. 6). Taking account of the reversed sign of optical rotation of dapiramicin B and the absence of *C*-methyl signal, the structure of dapiramicin B was tentatively considered to be 2-[4′-(4″-*O*-methyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]amino-5-cyano-4-methoxy-7*H*-pyrrolo[2,3-d]pyrimidine as shown in Fig. 4. The details of the structure deter-

mination of dapiramicin B will be reported elsewhere.

Biological Characterization

Dapiramicin A, at 1,000 μg/ml, showed no antibacterial or antifungal (yeast) activity. The minimum inhibitory concentration (MIC) of dapiramicin A against phytopathogenic fungi was determined by the agar dilution method, and the results are given in Table 2. It showed no significant *in vitro* antifungal activity. However, as shown in Table 3, dapiramicin A exhibited strong *in vivo* activity against the sheath blight of rice plants caused by *Rhizoctonia solani* in a green house test with no phytotoxicity to the rice plants (validamycin[4~7] was used as control). Epidapiramicin A and dapiramicin B were less effective. Therefore, the α-configuration of the glucopyranosylamine bond seems to be necessary for biological activity. On the other hand, dapiramicin A was less effective than validamycin in field tests.

Further, dapiramicin A was effective against the blast of rice plants caused by *Pyricularia oryzae* and the anthracnose of cucumber caused by *Colletotricum lagenarium* in green house tests[8].

Acute toxicity of dapiramicin A was examined using JCL-ICR mice (male, 5 week-old), and no mouse died upon administering the antibiotic at a dose of 200 mg/kg intravenously or 500 mg/kg intraperitoneally.

Table 2. Effect of dapiramicin A on phytopathogenic fungi *in vitro*.

Test organisms	MIC* (μg/ml)
Pellicularia filamentosa	100
Rhizoctonia solani	400
Pyricularia oryzae	>400
Diaporthe citri	>400
Colletotricum lagenarium	>400
Alternaria kikuchiana	>400
Glomerella cingulata	>400
Botrytis cinerea	>400
Fusarium oxysporum f. *lycopersici*	>400
Gibberella fujikuroi	>400
Cochliobolus miyabeanus	>400

* The MICs were determined on Czapek medium by the agar dilution method.

Table 3. Effect of dapiramicins on sheath blight of rice plants in a green house test.

Concentration (μg/ml)	Protective value (%)			
	Validamycin	Dapiramicin A	Epidapiramicin A	Dapiramicin B
100	98	96	48	45
50	97	93	35	34
25	86	89	31	28
12.5	40	59	21	29

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