NOVEL QUINOMYCINS: BIOSYNTHETIC REPLACEMENT OF THE CHROMOPHORES

Sir:

In general, certain amino acid residues in a heterodetic depsipeptide antibiotic are easily replaced by an analogous amino acid, *e. g.* methylproline in actinomycin[1] and isoleucine in quinomycin[2]. As previously reported by YOSHIDA[3], quinoxaline-2-carboxylic acid (QXA), the chromophore residue of quinoxaline antibiotics, was found to be an obligatory intermediate for the biosnythesis of these antibiotics by *Streptomyces*. These results led the authors to attempt to replace the chromophore of quinomycin by a structural analogue of QXA. In this communication, quinaldinic acid (QNA) was used as an analogue.

Streptomyses 731-I, which was isolated from *Streptomyces* sp. 732[4] by a colony selection technique and produced exclusively quinomycin A (echinomycin), was used as a quinomycin producer. The cultural conditions were as previously described except that the synthetic medium contained sodium nitrate instead of ammonium nitrate[2].

When QNA was supplemented into the medium at the time of inoculation at the concentrations given in Table 1, the synthesis of antibiotic was stimulated, maximal yield was attained with 1 mM of QNA. The antibiotic was extracted from the mycelium with acetone and from the filtrate with ethyl acetate. After removal of acetone from the mycelium extract, the antibiotic was transferred to ethyl acetate. The ethyl acetate extracts were combined, washed with a quater volume of 2 % sodium bicarbonate solution and concentrated to dryness under reduced pressure. The crude materials were purified by chromatography on silicic acid (Mallinckrodt, 100 mesh) with chloroform-methanol (99 : 1) elution. The concentration of the antibiotic was determined photometrically by the optical density at 240 mμ and the eluates containing the antibiotics were examined by thin layer chromatography on a plate of silica gel G (Merck) with methyl ethyl ketone. Under an ultraviolet lamp, two unknown spots with a bright fluorescence (Rf 0.70 and Rf 0.52) were detected besides an absorbing

Table 1. Effect of QNA on quinomycin formation by *Streptomyces* 732-I

QNA* (mM)	Antibiotic yield** (mcg/ml)
0	12
0.3	52
1.0	68
3.0	51
10.0	0

* QNA was added at the time of inoculation.
** The yield was measured photometrically after 7 days on the basis of the molecular absorbance ($\varepsilon_{243m\mu}$=67,500) of quinomycin A.

Table 2. Physicochemical characteristics of novel derivatives of quinomycin A

	QN-Quinomycin A	NX-Quinomycin A
Melting point	204.0°C	214.5°C
Specific rotation $[\alpha]_D^{25}$ in $CHCl_3$	−304.9°±7.1 (*c* : 0.507)	−303.9°±3.6 (*c* : 0.963)
λ_{max} in MeOH, mμ	239.5 (ε 84,200), 292, 313-6	240 (ε 67,100), 317
Molecular formula	$C_{52}H_{62}O_{12}N_{10}S_2$	$C_{51}H_{61}O_{12}N_{11}S_2$
Mol. weight* (Osmometry)	1,137(1,082)	1,114(1,083)
Analysis %* : C	57.52(57.67)	55.73(56.51)
H	5.98(5.73)	5.74(5.63)
N	12.65(12.94)	13.77(14.22)
S	6.57(5.92)	5.92(5.91)
Hydrolysates*(moles) D-Serine	1.76(2)	1.80(2)
L-Alanine	2.00(2)	2.00(2)
N-Methyl-L-valine	1.73(2)	2.04(2)
QXA	0.00(0)	1.19(1)
QNA	1.70(2)	1.28(1)
Paperchromatograph**, Rf	0.72	0.29

* The number in the bracket represents theoretical values.
** *n*-Butylether - sym. tetrachloroethane-10 % *o*-cresotinate (2 : 1 : 3), Toyo No. 50.

spot (Rf 0.34) which was identified with quinomycin A. The fractions containing these unknown substances were collected and concentrated to dryness. The residues were taken into a small volume of chloroform and separated preparatively by thin layer chromatography with the same system. The separated zones were individually scraped off, packed into small column and eluted with chloroform - methanol (5 : 1). Each material was recrystallized as needles from chloroform-methanol. Some physicochemical properties of these crystals are listed in Table 2. These materials were hydrolysed in 6 N HCl at 105°C for 40 hours. The hydrolysates were extracted with ethyl acetate, from which the chromophore of the antibiotics was recovered and purified by paper chromatography with system methanol - benzene - butanol - water - 28 % ammonia (40 : 20 : 20 : 20 : 1). The contents of QXA and QNA were determined photometrically (QXA : $\varepsilon_{239m\mu}$ 33,800, QNA : $\varepsilon_{234m\mu}$ 39,800 in methanol). When the aqueous solutions of the hydrolysates were analysed by a Hitachi amino acid analyser, the amino acid contents were identical with those of quinomycin A as listed in Table 2. On the basis of these results, it was determined that QXA residues in quinomycin A were substituted by two and one moles of QNA, in the fast and slow-moving substances

Fig. 1. Infrared spectra of QN-quinomycin A (top) and NX-quinomycin A (bottom) (Nujol)

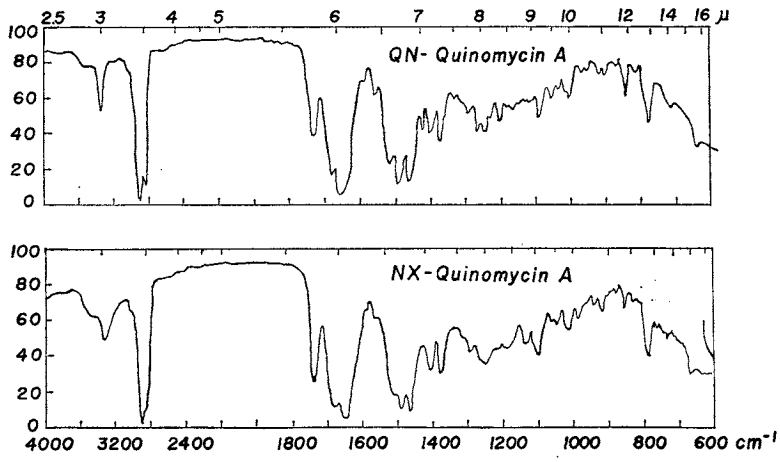

Table 3. Antibacterial spectra of novel derivatives of quinomycin A

Test organisms*	M. I. C. (mcg/ml)		
	QN-Quinomycin A	NX-quinomycin A	Quinomycin A
Bacillus anthracis	0.2	0.5	0.5
Bacillus subtilis, PCI-219	0.1	0.05	0.1
Staphylococcus aureus, 209P	0.2	0.2	0.2
Sarcina lutea	0.1	0.05	0.1
Diplococcus pneumoniae I**	0.05	0.02	0.05
Streptococcus hemolyticus, HA**	0.1	0.05	0.1
Corynebact. diphtheriae, S**	0.05	0.02	0.05
Staphylococcus aureus, 209P**	0.2	0.1	0.2
Shigella dysenteriae	1.0	2.0	5.0
Salmonella typhi	>50	>50	>50
Escherichia coli, UMEZAWA	20.0	20.0	20.0
Mycobacterium sp., 607	2.0	5.0	50.0
Mycob. phlei	2.0	5.0	10.0
Mycob. tuberculosis, $H_{37}Rv$	0.5	1.0	2.0

* Agar streak method. ** Blood agar.

respectively. The former substance was tentatively designated as QN-quinomycin A and the latter was NX-quinomycin A. These replacement of the chromophore of quinomycin A were reflected by the appearance of a specific quinoline ring absorption (1,565 and 1,593 cm^{-1}) as shown in Fig. 1. The antibacterial activities of QN-quinomycin A and NX-quinomycin A were compared with that of the parent antibiotic, quinomycin A (Table 3). These new antibiotics have activity against gram-positive bacteria virtually identical to quinomycin A. It is of interest that acid fast bacteria are more sensitive proportion to the content of QNA in the molecule. This diverse effect against different organisms may be due to an increased lipophilicity caused by the substitution. QN-Quinomycin A, NX-quinomycin A and quinomycin A at 0.01 μg/ml inhibited HeLa S_3 cells in suspension culture to the extent of 76%, 87% and 96%, respectively. QN-Quinomycin A and NX-quinomycin A were found to be effective against EHRLICH ascites carcinoma in mice with treatment by intraperitoneal injections once daily for 5 days of 0.4 mg/kg and 0.2 mg/kg, respectively, although the chemotherapeutic index is as low as 2, like that of quinomycin A. The LD_{50} of QN-quinomycin A, NX-quinomycin A and quinomycin A were 1.68, 0.84, and 0.28 mg/kg of mice, respectively, twenty days after a single intraperitoneal injection.

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